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High Initial Titres of Anti-Spike Antibodies following SARS-CoV-2 Infection is Associated with Faster Decay Rates at Four Months Follow-Up

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Abstract

Objective

Dynamics of humoral immune responses to SARS-CoV-2 antigens following infection suggests an initial decay of antibody followed by subsequent stabilization. We aim to understand the longitudinal humoral responses to SARS-CoV-2 nucleocapsid (N) protein and spike (S) protein and to evaluate their correlation to clinical symptoms among healthcare workers (HCW).

Design

A prospective cross-sectional cohort study.

Setting

This study was conducted in New York City Public Hospital in the South Bronx, New York.

Participants

Healthcare Workers participated in Phase 1 (N=500) and Phase 2 (N=178) of our study and underwent both PCR and serology testing, in addition to online survey. Analysis was performed on the 178 participants that presented for both phases of the study.

Primary outcome measure

Data from both phases over four months was collected on HCW that underwent serial qualitative serology testing for anti-N antibody, quantitative MSH-ELISA to detect Receptor Binding Domain and full-length S reactive antibodies to measure the decay rate and stabilization of the titres for SARS-CoV-2 infection.

Results

Anti-N antibody positivity was 27% and anti-S positivity was 28% in Phase 1. In Phase 2 anti-S titres were higher in symptomatic than in asymptomatic positive subjects in Phase 1. Marginally higher titers were seen in asymptomatic compared to the symptomatic positive subgroup in Phase 2. A positive correlation was noted between age, number and duration of

symptoms, and Phase 1 anti-S antibody titre. A strong correlation was observed between Phase 1 titers and decay of anti-S antibody titres between the two phases. Significant correlation with rate of decay was also noted with fever, GI symptoms, and total number and duration of COVID-19 symptoms.

Conclusions

Higher initial anti-S antibody titres were associated with larger number and longer duration of symptoms as well as faster decay during the two time points.

Strengths and limitations of this study

- The study captures the exposure risk for Healthcare Workers practicing during a pandemic and the antibody levels due to exposure to SARS-CoV-2 infection.
- In this cohort study that included 178 healthcare workers, over a 4-month period following the COVID-19 pandemic, participants had an initial rise in anti-nucleocapsid (N) and anti-spike (S) antibodies, which was followed by decay and stabilization of the titres.
- This study is limited by the single institutional data obtained from epicentre of the COVID-19 pandemic and the possibility of recall bias to the responses on the online survey may exist
- Another limitation of the study was that for Phase 2 a smaller number of participants followed up due to the Healthcare Workers who volunteered from around the country were transferred back and lost to follow-up.

Introduction

In light of the unprecedented coronavirus disease 2019 (COVID-19) pandemic, understanding the role of the immune system in countering the viral infection is critical not just to design effective antiviral strategies but also to aid us in taking appropriate public health decisions. The early publication of the viral genome led to a rapid development of many nucleic acid based diagnostic assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections. While nucleic acid-based tests are widely employed in the diagnosis of acute (current) SARS-CoV-2 infections, they are often limited in their clinical utility in identifying past infections or assess the level of immunity to SARS-CoV-2 within the communities. Evaluation of antibody responses is the other well-known modality used in a clinical setting that can detect both current, and past infections and is the preferred approach for surveillance to determine the true prevalence of infections. The currently available serological assays for SARS-CoV-2 target either the viral nucleoprotein (N) or the spike surface protein (S) antigens. The S-protein, which contains the receptor binding domain (RBD), binds to host cells via the angiotensin converting-enzyme-2 (ACE2) receptor, followed by membrane fusion[1,2]. The spike is the target of most neutralizing antibodies [3-5], while the N plays an important role in transcription enhancement and viral assembly [6]. Studies have demonstrated that antibodies against the N and S appeared around the same time - between day 8 and day 14 after the onset of symptoms with antibodies to the N being more sensitive than anti S antibodies for detecting early infection[7]. Neutralizing antibodies confer protective immunity and can be detected in most infected individuals 10-15 days following the onset of COVID-19 symptoms and remain elevated following initial viral clearance [8-12]. There is compelling evidence suggesting that serological assays for anti-S antibodies predict neutralizing activity, in contrast to N based assays[11,13].

The detailed characterization of the dynamics of humoral immune responses to the SARS-CoV-2 viral antigens following infection is still ongoing and early evidences suggest an initial decay of antibody followed by stabilization at a certain level[11,14-18]. These dynamics are likely driven by an initial expansion of plasmablasts which produce large amounts of antibody but die off quickly followed by a slower decay of antibody titres (the half-life of IgG is approximately three weeks) which then transitions into a steady state level of antibody produced by long-lived plasma cells[19]. However, it is currently unknown, if the magnitude of the initial expansion of plasmablast and the associated antibody titres are correlated with the steady state level of serum antibody produced by long-lived plasma cells. This is an important question since steady state antibody levels may provide superior protection from re-infection[20,21].

Specifically, there is currently a paucity of information on the kinetics of antibody decay among health care workers (HCW). It is suspected that SARS-CoV-2 infections among HCW are usually asymptomatic or mildly symptomatic and frequently associated with either underreporting of symptoms or heterogenous PCR and/or serologic diagnostics leading to most of them going undetected or unrecognized[22]. A large cohort study of HCWs in the greater New York City (NYC) area showed a seroprevalence of SARS-CoV-2 antibodies of 13.7%[23]. Our own data of anti N antibody screening among HCW at a New York City public hospital in the Bronx following the first "surge" of COVID-19 in May 2020, found that SARS-CoV-2 seroprevalence was at 27%[24]. Understanding the longitudinal kinetics of SARS-CoV-2 antibody response and the effectiveness of commercial antibody measurement assays is crucial to correctly determine infection rates, sero-prevalence and true sero-reversion rates in both

infected and vaccinated individuals – and to better understand protection associated with sero-positivity.

In this study, we aimed to investigate the longitudinal humoral responses to viral N and the spike and to evaluate their correlation to clinical symptoms and baseline characteristics in our HCW study cohort. Importantly, having access to samples during the initial antibody peak and several months out, we also aimed to determine if initial high antibody levels correlated with high antibody titers at steady state.

Methods

Study setting and population

The study is a prospective cross-sectional cohort study done in two phases after receiving Institutional Review Board approval (IRB # 20-009). The Phase 1 was conducted in May/June, 2020 and the Phase 2 was completed August/September 2020. The cohort included HCWs who worked at the New York City Public Hospital in the South Bronx and were willing to participate in both phases of the study. In the Phase 1 of the study, after informed consent, participants underwent qualitative serology testing (Abbott Architect SARS-CoV-2 IgG Assay, Abbott Park, IL 60064 USA)[25] and a nasopharyngeal swab for SARS-CoV-2 (Bio-Reference Laboratories, Inc., Elmwood Park, NJ, USA). They also completed an initial online survey on demographics, symptoms of COVID-19, healthcare/community exposure etc. An extra sample was collected and stored at -80°C for subsequent analysis. These samples were processed using a quantitative enzyme-linked immunosorbent assay (ELISA) that correlates well with virus neutralization, developed by Mount Sinai Health System (MSH ELISA)[26,27], to detect RBD and full-length spike (S) reactive antibodies. Participants from Phase 1 who agreed to return

for follow up serology testing (Abbott and MSH ELISA) and completion of a follow-up online survey were part of Phase 2 of the study.

Antibody assays

The Abbott Architect assay uses a qualitative chemiluminescent microparticle immunoassay technology targeting the N antigen of the virus with a reported sensitivity of 100% (CI 95.8–100%) and specificity of 99.6% (CI 99–99.9%)[25]. The MSH ELISA consists of an initial ELISA using serum or plasma to detect specific IgG against the RBD of SARS-CoV-2 at a single dilution, followed by quantitative titrations of presumptive positives in a confirmatory ELISA against full length SARS-CoV-2 spike protein (S)[28]. The positive result from the spike ELISA is reported as antibody at a titre of 1:80 or higher. Test performance assessment revealed that PCR+ samples were 94 % positive and all negative samples returned a negative result for 100% negative agreement[29].

Survey

The online survey was accessed by a unique identification number assigned to each participant, blinded to the research team to ensure confidentiality. The survey requested information on age, race/ethnicity, comorbidities, domestic/international travel and healthcare and community exposure details during and prior to both phases. The first phase collected information about symptoms of COVID-19 including their timing and duration in the preceding weeks of the blood draw[24]. The Phase 2 survey requested information on new comorbidities, persistent COVID-19 symptoms (cough, shortness of breath, anosmia, ageusia, myalgia, nausea, and/or diarrhea), interim testing via antibody and/or reverse transcription polymerase chain reaction (RT -PCR) (if present) and their result (positive/negative), presence of positive SARS-CoV-2 PCR results in the preceding months, interim domestic/international travel and continued use

of personal protective equipment (PPE). The risk of exposure in the healthcare setting and community exposure was determined based on CDC guidelines[30].

Statistical analysis

Convenience sampling design was adapted to recruit participants with a goal of 500 participants. Descriptive statistics were used to summarize the baseline characteristics of the cohort and key study outcome variables. Categorical variables were compared by the Chi–squared test, while continuous variables were compared by a Student's t-test. The spike antibody titres were described as geometric means. Correlations were calculated using standard Pearson and Spearmen correlation. Multiple linear regression was applied to determine the predictors of log10 rate of decay from Phase 1 to Phase 2 of anti-spike antibodies. A p-value of <0.05 was considered significant. All statistical analyses were performed using SPSS version 27 (IBM, USA).

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Results

For Phase 1 of our study, 500 healthcare workers underwent both PCR and serology testing. Of these, 137 were positive by for anti-N antibody (Abbott) and 142 were positive by the MSH ELISA. For the second phase 178 participants from the initial cohort consented and underwent evaluation with PCR, antibody assays (Abbott and MSH ELISA) and completed the online follow up survey. The details of patient enrolment are described in **Figure 1**. While 46 of the 178 tested subjects remained positive for the anti-N antibody (Abbott), 70 were positive by the MSH ELISA in the second phase. Anti -spike titres of the 5 subjects in the first phase were close to the cut off for positivity. Twenty-two subjects who were negative for anti-N antibody

in Phase 2 had positive titres of anti-RBD and anti-spike antibodies, though lower than their Phase 1 levels. Among the subjects who participated in the Phase 1 and Phase 2 study, 68 were positive in both phases by the MSH ELISA, 110 were negative in both phases and 2 were positive only in Phase 2 with previously negative results in Phase 1.

The baseline characteristics of study participants who were positive by MSH ELISA in both phases (n=68) and those who were negative in both phases (n=108) are shown in **Table 1**. The mean age of the participants was 44.7±12.4 years, and 63.1% were female. Overall, 30.7% of the HCWs were Latinx, 29.5% were Asian, 16.5% were Black and 17.6% were White. COVID-19 related symptoms were present in 83.8% (57) of the subjects who were positive in both phases, while only 42.6% (46) of the subgroup who had negative antibodies in both phases admitted to symptoms prior to Phase 1. The duration of symptoms prior to Phase 1 was longer among the symptomatic positive group (48.3% for >14 days) in comparison to symptomatic negative group (17.8% for >14 days). The mean duration of symptoms to Phase 1 testing in the symptomatic positive sub cohort was 47.9 ±16.0 days. Persisting symptoms of COVID-19 were reported in 19 (27.9%) subjects from the cohort with positive antibodies in both phases.

Clinical characteristics and seropositivity to spike protein in both phases

Table 2 describes the characteristics of the symptomatic and asymptomatic subjects who were positive for anti-spike antibody in both phases. Baseline characteristics were comparable between the groups and no difference either in the healthcare or community exposure or in the location of work (ED/Inpatient/intensive care unit, OR etc.) between the two groups was observed. Titres of anti-spike antibodies (geometric mean area under the curve (AUC)) were higher in symptomatic subjects than in asymptomatic positive subjects (6754 AUC vs. 5803 AUC) in Phase 1. However, in the Phase 2 analysis we observed marginally higher titres in the

asymptomatic subgroup compared to the symptomatic subgroup (2383 AUC vs. 2198 AUC). The rate of decay was higher in the symptomatic subgroup (geometric mean 32.96 per day) compared to the asymptomatic (geometric mean 23.42 per day) suggesting delayed antibody/kinetics in the asymptomatic cohort.

Phase 1 anti-spike antibody titre and clinical correlations

A Pearson's product and Spearman's rank-order correlation was run to assess the relationship between cohort characteristics including age, gender, comorbidities, number of symptoms of COVID-19, healthcare exposure and Phase 1 anti-spike titres in our cohort (**Figure 2**). One hundred-forty-three subjects with a positive test in Phase 1 were included in the analysis. Scatter plot analysis showed a monotonic relationship between the variables. A statistically significant weak positive correlation was observed between age and Phase 1 anti-spike antibody titres ($R=0.269$, $p<0.005$). Moderate positive correlation was present between presence of fever ($R=0.319$, $p<0.005$), number of symptoms ($R=0.310$, $p<0.005$) and days of symptoms ($R=0.434$, $p<0.005$) and anti-spike antibody titre; and weak positive correlation was observed with upper respiratory symptoms ($R=0.278$, $p<0.005$) and gastrointestinal (GI) symptoms ($R=0.204$, $p<0.05$) with anti-spike antibody titres.

Correlation of rate of decay of anti-spike antibody titres from Phase 1 to Phase 2 and clinical characteristics

Results of Pearson correlation to assess the relationship between cohort characteristics including Phase 1 anti-spike antibody titres, age, gender, comorbidities, symptoms of COVID-19, number of symptoms of COVID-19, healthcare exposure and decay of anti-spike titres between the two phases in our cohort is shown in **Figure 3.** A strong positive statistically significant correlation was observed between Phase 1 titers and decay of anti-spike antibody

titres between the two phases (R=0.898, p<0.000). Medium positive correlation was observed between presence of fever (R=0.428, p<0.001), GI symptoms (R=0.340, p<0.011), number of symptoms (R=0.357, p<0.007), duration of symptoms (R=0.469, p<0.000) with decay of anti-spike antibody titres between the two phases respectively.

A pairwise comparison was performed between rate of decay of anti-spike antibody titres and patient characteristics (**Figure 4**). Rate of decay by gender was comparable (male; 30.73 AUC/day vs. female;34.68 AUC/day, p=0.413). Asian (86.0 AUC/day) race showed higher rate of decay compared with White (7.2 AUC/day) and Black (19.61 AUC/day) individuals; while Latinx (47.28 AUC/day) race had higher rate of decay compared with White (7.2 AUC/day) individuals. Subjects with fever had a higher rate of decay than those who did not report fever (53.08 AUC/day vs.16.14 AUC/day, p=0.002). Similarly subjects with GI symptoms had a higher rate of decay than those without (55.81 AUC/day vs.21.94 AUC/day, p=0.019). Subjects with symptoms restricted to less than seven days demonstrated a lower decay rate compared with symptomatic subjects over 7-14 days (13.60 AUC/day vs. 36.12 AUC/day, p=0.046) and when compared with symptomatic subjects with more than 14 days (13.60 AUC/day vs. 59.72 AUC/day, p=0.001). This finding was statistically significant. No difference was found when degree of exposure (High/Moderate: 28.18 AUC/day vs. Mild: 34.78 AUC/day, p=0.395) or job role (physician: 29.57 AUC/day vs. nurse: 53.59 AUC/day vs. Other: 26.83 AUC/day; p=0.361) was compared to rate of decay.

Predictors of rate of decay from Phase 1 to Phase 2 of anti-spike antibodies

Multiple linear regression analysis to predict the rate of decay with respect to age, Bacillus Calmette Guerin vaccination, number of symptoms, and Phase 1 (log10) anti-spike antibody titres is shown in **Table 3**. On the basis of a linear regression model that included the

participants age, history of BCG vaccination, total number of COVID-19 symptoms and the Phase 1 concentration of log 10 spike antibody titres, the estimated change (decay) was 23.6 AUC/day when age was centred at median (42.6 years), there was positive history of BCG vaccination, the total number of COVID-19 symptoms were centred at a median of 4, and the geometric mean of the $\log_{10}$ spike antibody titre was 3.78.

Discussion

With the COVID-19 pandemic showing no signs of abating, healthcare workers at the epicentre are at risk of infection due to occupational exposure as well as community exposure. Sero-surveillance is the foundation for determining the scale and rate of exposures. With a multitude of serological assays getting emergency use approval from FDA, interpretation of the results of these assays and their clinical significance remains challenging. It is critical to understand the timing of the antibody response for acute interpretation. Confidence in analytical specificity of the assay is a critical requirement in measurement of the specific antibody responses. Recent studies have confirmed that anti spike titres especially anti-RBD titres can serve as surrogates for virus neutralization[31,32]. The Abbott SARS-CoV-2 IgG assay that targets antibodies to the nucleoprotein has a reported specificity and sensitivity of greater than 99% at 14 days or more following symptom onset and these measurements are not indicative or correlated to virus neutralization titres[33]. In comparison, the MSH ELISA targets the full-length S protein including RBD, a major target for neutralizing antibodies and has demonstrated excellent correlation to virus neutralization[11,26]. Longitudinal measurements of antibody levels have revealed that anti-N and anti S IgG antibodies continue to increase until the third week post symptoms and an approach that combines the detection of both of these antibodies would precisely detect almost 100% of all infectious exposures[34]. In our study, the mean number of days after

symptoms to testing in Phase 1 was 47 days suggesting a higher likelihood of accuracy of the utilized assay.

Longitudinal blood sampling among HCWs working at a public hospital in the epicentre of the pandemic in NYC allowed for analysis of kinetics of anti-S and anti-N antibody responses. At two months after the first surge of infections, anti-N antibodies were detected in 27% and anti-S antibodies in 28% of participating HCWs. After an interval of four months, it is not surprising to note that among the participants who returned, 26% remained positive for anti N antibodies, while 31% of the previously anti-N antibody positive subjects tested negative in phase 2. On the other hand, a similar analysis of the anti-S antibodies levels, confirmed that all the previously positive retested subjects continued to remain positive, albeit with lower titres.

COVID-19 related symptoms were significantly associated with positive anti-spike antibodies in both phases, with a similar association with longer duration (>14 days) of symptoms. Previous studies have demonstrated a lower level of IgG response among patients without symptoms or with mild symptoms compared to those with severe and critical disease[35,36]. A comparison of symptomatic versus asymptomatic subjects who tested positive for anti-spike antibodies in both phases, confirmed that the rate of decay of anti-spike antibody titres were faster in the symptomatic cohort than the asymptomatic subjects, which was seen also in the anti-N antibody kinetics. However, we observed a faster decay in this group with a lower titre of anti-spike antibodies in Phase 2 compared to the asymptomatic cohort (though the difference was not statistically significant). This could additionally be supported by the finding of fever and GI symptoms contributing to faster decay. Similar results of decreasing neutralizing antibody titre in symptomatic than asymptomatic patients were observed by Choe *et al.*[37]

Positive correlations for age, presence of fever, upper respiratory symptoms, GI symptoms, total number and duration of symptoms was observed with increased levels of anti-spike titres at Phase 1. Similar results of neutralizing antibody titres were also observed by Boonyaratanakornkit *et al.* wherein they showed higher levels of neutralizing antibody titres were significantly associated with male gender, older adults, higher disease severity and shorter interval from recovery[38]. Based on a linear regression model with age centred at median (42.6 years), positive history of BCG vaccination, the total number of COVID-19 symptoms centred at a median of 4, and the geometric mean of the log10 anti-spike antibody titre at 3.78, we observed that the rate of decay of these antibody titres was 23.6 AUC/day. Evaluation of other characteristics with rate of decay between Phase 1 and Phase 2 showed a faster reduction in titres in Asian participants and in those with fever and GI symptoms. A slower decrease was noted among patients with shorter duration (<7 days) of symptoms, with no other significant correlation noted with any other baseline demographics or clinical characteristics.

As described above, higher antibody titers are associated with a larger number of symptoms, longer duration of symptoms and – as described by others as well – disease severity in general. We also found that higher initial antibody titers were associated with faster antibody decay during the two time points. Initial antibody responses are driven by short lived plasmablasts, which decay after a few days after producing massive amounts of antibody. IgG has a relatively long half-life of approximately three weeks, but decay is inevitable since the plasmablasts initially producing it disappear. Usually, titers then drop until they reach relatively stable levels of antibody which are maintained by long-lived plasma cells in the bone marrow[19]. The two time points described in this study represent the initial peak response and likely the stable level after the initial decay. We found that individuals with higher initial titers had a faster decay rate during the observation period meaning the difference between peak and stable, long-lived

antibody levels were larger. This indicates that there is likely no direct correlation between the magnitude of the initial expansion of plasmablasts and the number of long-lived plasma cells that migrate to the bone marrow.

Our study has the following limitations: First being a single center study with a small convenience sampling that included a smaller number of participants in Phase 2 of the study. Following the pandemic, the HCWs who volunteered from around the country were transferred back and lost to follow-up, which did decrease the overall sample size, but the rates of positive and negative results remained proportional. Second, the likelihood of a recall bias in the participant's responses on the online survey may exist. Lastly, as a cross-sectional seroprevalence study the findings can underestimate rates of prior infections based on timing of the testing given that antibodies are only transiently detectable following infection.

In conclusion, findings from this study are similar to other studies that have reported that higher magnitude of anti-spike titres may correlate with protection against reinfection, in spite of the observed decay in the antibody levels[20,21]. Nevertheless, further studies to evaluate the longevity of immunity, especially in context of widespread administration of spike-based vaccine among HCWs would be important in predicting herd immunity to COVID-19 infections.

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Deceased: Author Bo Yu MD has deceased and will always be remembered.

Contributions: V.M., M.A.S. and U.V. designed the study. J.M.C., M.A.S., B.Y., V.P.G. analysed the data. E.V. and M.G. assisted with participant follow-up and coordination with the assistance of A.P., U.V., V.M., and M.A.S. The Mount Sinai Health System team, J.M.C. and F.K., performed the measurements for anti-Spike and Anti-RBD antibodies. V.M., U.V. and A.P. were responsible for the clinical care of the research participants and supervised the day-to-day operation and coordination of the study by M.K., V.D., M.A.S., B.Y., V.P.G., M.G., E.V., and M.G.

V.M. and F.K wrote the manuscript and is the guarantor of this work and has full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis, with the assistance of J.C.Q., M.A.S., B.Y., V.P.G., and M.G.

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Competing Interests: F.K. is listed as a co-inventor on a patent application filed by The Icahn School of Medicine at Mount Sinai relating to SARS-CoV-2 serological assays and NDV-based SARS-CoV-2 vaccines. Mount Sinai has spun out a company, Kantaro, to market serological tests for

SARS-CoV-2. F.K. has consulted for Merck and Pfizer (before 2020), and is currently consulting for Seqirus and Avimex. F.K.'s Krammer laboratory is also collaborating with Pfizer on animal models of SARS-CoV-2.

All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Patient and public involvement: Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication: Not required

Ethics approval: The study protocol was approved by the Institutional Review Board approval (IRB # 20-009, Lincoln Medical Center, Office of the Institutional Review Board approved as per 45 CFR 46 & 21 CFR50,56 under a full board committee and gave its approval on 4/28/2020). All participants provided written informed consent for the use of their data.

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Table 1: Broad characteristics among health care workers assessed for antibody reactivity to spike SARS-CoV-2 protein in Phase 1 and Phase 2

	Overall †	Spike ELISA (AUC) positive in both phases	Negative Reactivity to spike (AUC) in both phases	p value
	N=176	n=68	n=108	
Age, years	44.7+12.4	42.9+11.9	45.8+12.7	0.099
Female, Gender	111 (63.1%)	40 (58.8%)	71 (65.7%)	0.467
Race				0.666
Latinx	54 (30.7%)	21 (30.9%)	33 (30.6%)	
Asian	52 (29.5%)	18 (26.5%)	34 (31.5%)	
Black	29 (16.5%)	15 (22.1%)	14 (13.0%)	
White	31 (17.6%)	10 (14.7%)	21 (19.4%)	
Other	10 (5.7%)	4 (5.9%)	6 (5.9%)	
Comorbidities	54 (30.7%)	25 (36.8%)	29 (26.9%)	0.214
BCG vaccine received in childhood	87 (49.4%)	35 (51.5%)	52 (48.1%)	0.902
COVID-19 related symptoms prior to Phase 1	103 (58.5%)	57 (83.8%)	46 (42.6%)	<.001
Duration of symptoms				<.001
<7 days	48 (46.6%)	18 (31.0%)	30 (66.7%)	
7-14 days	19 (18.4%)	12 (20.7%)	7 (15.6%)	
>14 days	36 (35.0%)	28 (48.3%)	8 (17.8%)	
Time from symptom to positive result, days	45.7+19.9	47.9+16.0	42.9+24.1	0.062
RT-PCR positive result for SARS-CoV-2 prior to Phase 1	51 (29.0%)	49 (72.1%)	2 (1.9%)	<.001
RT-PCR positive result for SARS-CoV-2 during Phase 1	14 (8.0%)	13 (19.1%)	1 (0.9%)	<.001
Persisting symptoms from COVID-19	25 (14.2%)	19 (27.9%)	6 (5.6%)	<.001
Nature of work				0.306
Physicians	81 (46.0%)	29 (42.6%)	52 (51.5%)	
Nurses	29 (16.5%)	15 (22.1%)	14 (13.0%)	
Others	64 (36.4%)	24 (35.3%)	40 (39.6%)	
Hospital areas worked in:				
Emergency department/Inpatient units	118 (67.0%)	50 (73.5%)	68 (63.0%)	0.141
Ambulatory care/Clinics	72 (40.9%)	27 (39.7%)	45 (41.7%)	0.631
Administration/Non-clinical care areas	24 (13.6%)	9 (13.2%)	15 (13.9%)	0.867
Community exposure	47 (26.7%)	19 (27.9%)	28 (25.9%)	0.591

Household exposure	39 (22.2%)	17 (25.0%)	22 (20.4%)	0.343
PPE use at work	173 (98.3%)	67 (98.5%)	106 (98.1%)	0.226
Use of facemask outside of the hospital	158 (89.8%)	58 (85.3%)	100 (92.6%)	0.062

Continuous variables are expressed as mean (SD), categorical variables as n (%).
BCG, Bacillus Calmette–Guérin vaccine; PPE, personal protective equipment; RT-PCR, reverse transcription polymerase chain reaction.
† Demographic data is missing for 2 participants from the overall cohort.

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Table 2: Broad characteristics among health care workers with positive antibody reactivity to SARS-CoV-2 spike in both phases

	Overall	Asymptomatic for SARS-CoV-2 infection	Symptomatic for SARS-CoV-2 infection	*p value*
	n=68	n=11	n=57	
Age, Mean (±SD)	42.9 (±1.45)	44.5 (±3.8)	42.6 (±1.6)	0.557
Female, n (%)	40 (58.8%)	6 (54.5%)	34 (40.4%)	0.502
Race				0.753
Latinx	21 (30.9%)	3 (27.3%)	18 (31.6%)	
Asian	18 (26.5%)	3 (27.3%)	18 (26.3%)	
Black	15 (22.1%)	3 (27.3%)	12 (21.1%)	
White	10 (14.7%)	2 (18.2%)	8 (14.0%)	
Other	4 (5.8%)	0 (0%)	4 (7.0%)	
Comorbidities				
Hypertension	13 (19.1%)	2 (18.2%)	11 (19.3%)	0.650
Diabetes	6 (8.8%)	0 (0%)	6 (10.5%)	0.332
COPD and asthma	13 (19.1%)	1 (9.1%)	12 (21.1%)	0.326
Number of symptoms, median (IQR)	-	-	4.0 (2.0-5.0)	
Length of symptoms				
<7 days	-	-	19 (33.3%)	
7-14 days	-	-	12 (21.1%)	
>14 days	-	-	26 (45.6%)	
Degree of HCW exposure				0.492
High and Moderate	16 (23.5%)	2 (18.2%)	14 (24.6%)	
Minor	52 (76.5%)	9 (81.8%)	43 (75.4%)	
Community exposure	19 (27.9%)	3 (27.3%)	16 (28.1%)	0.635
Household exposure	17 (25.4%)	3 (27.3%)	14 (24.6%)	0.557

Use of facemask outside of hospital	58 (85.3%)	9 (81.8%)	49 (86.0%)	0.722
Principal means of transportation				0.663
Public	33 (48.5%)	6 (54.5%)	27 (47.7%)	
Private	35 (51.5%)	5 (45.5%)	30 (52.6%)	
Nature of work				0.502
Physician	29 (42.6%)	4 (36.4%)	25 (43.9%)	
Nurse	15 (22.1%)	2 (18.2%)	13 (22.8%)	
Other	24 (35.3%)	5 (45.5%)	19 (33.3%)	
Hospital areas work in:				0.288
Emergency department/inpatient units	32 (47.1%)	6 (54.5%)	26 (45.6%)	
Ambulatory care/clinics	9 (13.2%)	2 (18.2%)	7 (12.3%)	
Impatient and outpatient setting	18 (26.5%)	3 (27.3%)	15 (26.3%)	
Administration/nonclinical care areas	9 (13.2%)	0 (0%)	9 (15.8%)	
Anti-spike reactivity (AUC)				
Reactivity in phase 1, G-Mean (IQR)	6590 (5165-8410)	5803 (2825-11920)	6754 (5177-8812)	0.647
Days from symptoms to first test, Mean (±SD)	-	-	47.7 (±1.9)	
Reactivity in phase 2, G-Mean (IQR)	2226 (1824-2718)	2382 (1494-3797)	2198 (1753-2755)	0.980
Days from symptoms to second test			174.5 (±4.1)	
Rate of decay, G-Mean (IQR)	31.14 (22.11-43.87)	23.42 (8.45-64.93)	32.96 (22.73-47.82)	0.382

Continuous variables are expressed as mean (SD) or interquartile range (IQR), categorical variables as n (%).

AUC, area under the curve; COPD, Chronic obstructive pulmonary disease; HCW, health care worker

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Table 3: Multiple linear regression analysis of rate of decay for anti-spike antibodies between Phase 1 and Phase 2

Rate of decay (log10)	*B*	95.0% CI for B		*SE B*	ß	R^2	▲R^2
		LL	UL				
Model						0.83	0.82
Constant	-3.203**	-3.647	-2.759	.222			
Age (per 10-year change)	.014	-.005	.007	.002	.030		
BCG vaccination	.131**	.030	.310	.046	.121		
Number of symptoms	.013	-.029	.060	.012	.050		
ELISA reactivity (Log10)	1.159**	1.050	1.419	.059	.916		

*B: Un*standardized regression coefficient; CI: confidence interval; LL: lower limit; UL: upper limit; SE B: standard error of the coefficient; ß: standardized coefficient; R^2: coefficient of determination; ▲R^2: adjusted R^2.

***P<0.*05

BCG, Bacillus Calmette–Guérin vaccine

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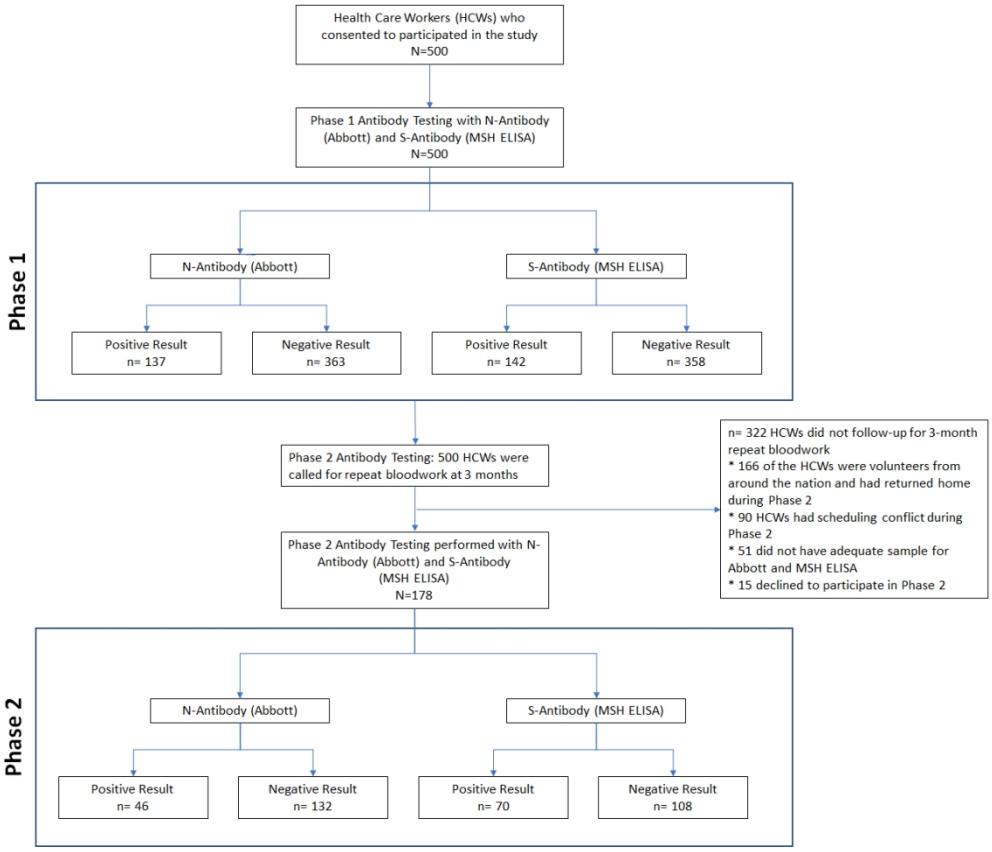
Figure 1: Flow Chart of patient enrollment, follow up and analysis

Figure 2: Simple correlation analysis of HCW with positive reactivity for anti- spike antibody in Phase 1 with baseline characteristics and symptoms

Figure 3: Simple correlation analysis of rate of decay of anti-spike antibodies between both phases with baseline characteristics and symptoms

Figure 4: Paired comparison between rate of decay of anti-spike antibody titres and patient characteristics



Flow Chart of patient enrollment, follow up and analysis

340x286mm (96 x 96 DPI)

		ELISA reactivity in Phase 1 (Log10)	Age	Gender	Comorbidities	BCG vaccine	Fever	URS	GI symptoms	Number of symptoms	Days of symptoms	Degree of HCW exposure
ELISA reactivity in Phase 1 (Log10)	R	1										
	Sig.	-										
Age	R	0.269**	1									
	Sig.	0.001	-									
Gender	R	0.106	0.050	1								
	Sig.	0.209	0.552	-								
Comorbidities	R	0.130	0.468**	0.045	1							
	Sig.	0.123	0.000	0.596	-							
BCG vaccine	R	0.046	0.014	-0.129	0.084	1						
	Sig.	0.585	0.869	0.125	0.317	-						
Fever	R	0.319**	0.024	-0.089	0.018	0.060	1					
	Sig.	0.000	0.777	0.292	0.828	0.479	-					
URS	R	0.278**	0.039	0.059	0.069	-0.214*	0.464**	1				
	Sig.	0.001	0.645	0.486	0.413	0.010	0.000	-				
GI symptoms	R	0.204*	0.030	0.068	0.029	0.024	0.344**	0.291**	1			
	Sig.	0.015	0.723	0.423	0.733	0.775	0.000	0.000	-			
Number of symptoms	R	0.310**	-0.015	0.070	0.026	0.019	0.708**	0.665**	0.630**	1		
	Sig.	0.000	0.856	0.405	0.762	0.825	0.000	0.000	0.000	-		
Days of symptoms	R	0.434**	0.036	0.019	0.121	-0.038	0.422**	0.605**	0.347**	0.681**	1	
	Sig.	0.000	0.671	0.817	0.151	0.656	0.000	0.000	0.000	0.000	-	
Degree of HCW exposure	R	-0.145	-0.215**	-0.217**	-0.177*	0.017	0.017	0.034	0.000	0.039	-0.085	1
	Sig.	0.083	0.010	0.009	0.034	0.838	0.844	0.690	0.998	0.640	0.314	-

BCG, Bacillus Calmette–Guérin; URS, upper respiratory symptoms; GI, Gastrointestinal symptoms (nausea, vomit and diarrhoea); HCW, health care worker.
**. Correlation is significant at the 0.01 level., *. Correlation is significant at the 0.05 level.

Simple correlation analysis of HCW with positive reactivity for anti- spike antibody in Phase 1 with baseline characteristics and symptoms

767x285mm (96 x 96 DPI)

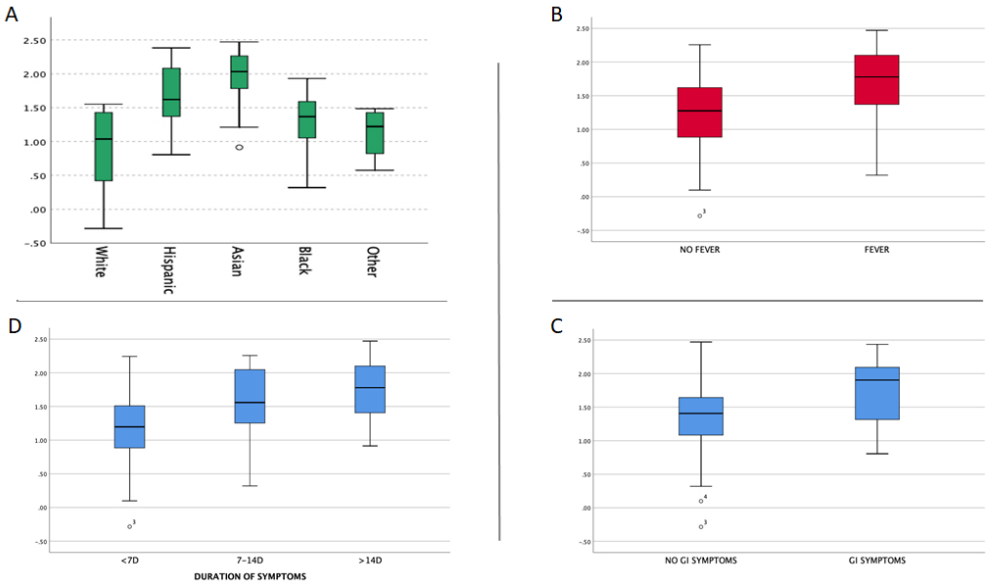
		Rate of decay (Log10)	ELISA reactivity (Log10)	Age	Gender	Comorbidity	Fever	GI symptoms	Number of symptoms	Duration of symptoms	Degree of HCW exposure
Rate of decay (Log10)	R	1									
	sig.	-									
ELISA reactivity (Log10)	R	0.898**	1								
	sig.	0	-								
Age	R	0.176	0.182	1							
	sig.	0.198	0.184	-							
Gender	R	0.044	0.094	0.001	1						
	sig.	0.751	0.493	0.994	-						
Comorbidity	R	0.193	0.156	0.514**	-0.092	1					
	sig.	0.158	0.257	0.000	0.502	-					
Fever	R	0.428**	0.463**	-0.090	-0.090	-0.122	1				
	sig.	0.001	0.000	0.515	0.512	0.374	-				
GI symptoms	R	0.340*	0.289*	-0.020	-0.072	-0.163	0.269*	1			
	sig.	0.011	0.033	0.885	0.603	0.234	0.047	-			
Number of symptoms	R	0.357**	0.360**	-0.256	0.008	-0.193	0.640**	0.555**	1		
	sig.	0.007	0.007	0.059	0.954	0.158	0.000	0.000	-		
Duration of symptoms	R	0.469**	0.469**	-0.049	0.123	0.142	0.204	0.207	0.430**	1	
	sig.	0.000	0.000	0.723	0.370	0.300	0.136	0.129	0.001	-	
Degree of HCW exposure	R	0.067	0.106	0.154	0.266*	0.202	-0.136	-0.075	-0.184	0.133	1
	sig.	0.625	0.439	0.261	0.050	0.140	0.321	0.586	0.180	0.334	-

BCG: Bacillus Calmette–Guérin; GI: Gastrointestinal symptoms (nausea, vomit and diarrhea); HCW: health care worker.

**. Correlation is significant at the 0.01 level, *. Correlation is significant at the 0.05 level

Simple correlation analysis of rate of decay of anti-spike antibodies between both phases with baseline characteristics and symptoms

686x269mm (96 x 96 DPI)



Paired comparison between rate of decay of anti-spike antibody titres and patient characteristics

270x161mm (96 x 96 DPI)

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	#3	State specific objectives, including any prespecified hypotheses	7
Methods			

Study design	#4	Present key elements of study design early in the paper	7
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	7
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	7
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	8
Bias	#9	Describe any efforts to address potential sources of bias	16
Study size	#10	Explain how the study size was arrived at	9
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	7-9
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	
9			
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	9
Statistical methods	#12c	Explain how missing data were addressed	9
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	9
Statistical methods	#12e	Describe any sensitivity analyses	

9			
Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	9
Participants	#13b	Give reasons for non-participation at each stage	30
Participants	#13c	Consider use of a flow diagram	
30			
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	9,23-24
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	
9,23-26			
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	
4			
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	
4			
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-11
Main results	#16b	Report category boundaries when continuous variables were categorized	10
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	

11			
Other analyses	#17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11-13
Discussion			
Key results	#18	Summarise key results with reference to study objectives	13-14
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	16
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	15-16
Generalisability	#21	Discuss the generalisability (external validity) of the study results	16-17
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

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Longitudinal Humoral Antibody Response to SARs-CoV2 Infection among Health Care Workers in a New York City Hospital

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Secondary Subject Heading:	Infectious diseases, Immunology (including allergy)
Keywords:	COVID-19, INFECTIOUS DISEASES, IMMUNOLOGY

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Longitudinal Humoral Antibody Response to SARS-CoV2 infection among Health Care Workers in a New York City Hospital

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Abstract

Objective

Dynamics of humoral immune responses to SARS-CoV-2 antigens following infection suggests an initial decay of antibody followed by subsequent stabilization. We aim to understand the longitudinal humoral responses to SARS-CoV-2 nucleocapsid (N) protein and spike (S) protein and to evaluate their correlation to clinical symptoms among healthcare workers (HCW).

Design

A prospective cross-sectional cohort study.

Setting

This study was conducted in New York City Public Hospital in the South Bronx, New York.

Participants

Healthcare Workers participated in Phase 1 (N=500) and Phase 2 (N=178) of our study and underwent both PCR and serology testing, in addition to online survey. Analysis was performed on the 178 participants that presented for both phases of the study.

Primary outcome measure

Data from both phases over four months was collected on HCW that underwent serial qualitative serology testing for anti-N antibody, quantitative MSH-ELISA to detect Receptor Binding Domain and full-length S reactive antibodies to measure the decay rate and stabilization of the titres for SARS-CoV-2 infection.

Results

Anti-N antibody positivity was 27% and anti-S positivity was 28% in Phase 1. In Phase 1 anti-S titres were higher in symptomatic (6754(5177-8812) than in asymptomatic positive subjects (5803(2825-11920). Marginally higher titers (2382(1494-3797) were seen in asymptomatic compared to the symptomatic positive subgroup (2198 (1753-2755) in Phase

2. A positive correlation was noted between age (R=0.269, p<0.005), number (R=0.310, p<0.005) and duration of symptoms (R=0.434, p<0.005), and Phase 1 anti-S antibody titre. A strong correlation (R=0.898, p<0.000). was observed between Phase 1 titers and decay of anti-S antibody titres between the two phases. Significant correlation with rate of decay was also noted with fever (R=0.428, p<0.001), GI symptoms (R=0.340, p<0.011), and total number (R=0.357, p<0.007) and duration of COVID-19 symptoms (R=0.469, p<0.000).

Conclusions

Higher initial anti-S antibody titres were associated with larger number and longer duration of symptoms as well as faster decay during the two time points.

Strengths and limitations of this study

•The strength of our study is the longitudinal design with serial sampling to determine humoral response to SARS-CoV-2 infection from consenting Health Care Workers during the pandemic.

•This study collected serial detailed characteristics of symptomatic and asymptomatic Health Care Workers to correlate with durability and decay of humoral response.

•This study is limited by the single institutional data obtained from epicentre of the COVID-19 pandemic and the possibility of recall bias to the responses on the online survey may exist

•Our cohort for Phase 2 was smaller than Phase 1, due to discontinuation of volunteer healthcare workers from the surge period."

Introduction

In light of the unprecedented coronavirus disease 2019 (COVID-19) pandemic, understanding the role of the immune system in countering the viral infection is critical not just to design effective antiviral strategies but also to aid us in taking appropriate public health decisions. The early publication of the viral genome led to a rapid development of many nucleic acid based diagnostic assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections. While nucleic acid-based tests are widely employed in the diagnosis of acute (current) SARS-CoV-2 infections, they are often limited in their clinical utility in identifying past infections or assess the level of immunity to SARS-CoV-2 within the communities. Evaluation of antibody responses is the other well-known modality used in a clinical setting that can detect both current, and past infections and is the preferred approach for surveillance to determine the true prevalence of infections. The currently available serological assays for SARS-CoV-2 target either the viral nucleoprotein (N) or the spike surface protein (S) antigens. The S-protein, which contains the receptor binding domain (RBD), binds to host cells via the angiotensin converting-enzyme-2 (ACE2) receptor, followed by membrane fusion[1,2]. The spike is the target of most neutralizing antibodies [3-5], while the N plays an important role in transcription enhancement and viral assembly [6]. Studies have demonstrated that antibodies against the N and S appeared around the same time - between day 8 and day 14 after the onset of symptoms with antibodies to the N being more sensitive than anti S antibodies for detecting early infection[7]. Neutralizing antibodies confer protective immunity and can be detected in most infected individuals 10-15 days following the onset of COVID-19 symptoms and remain elevated following initial viral clearance [8-12]. There is compelling evidence suggesting that serological assays for anti-S antibodies predict neutralizing activity, in contrast to N based assays[11,13].

The detailed characterization of the dynamics of humoral immune responses to the SARS-CoV-2 viral antigens following infection is still ongoing and early evidences suggest an initial decay of antibody followed by stabilization at a certain level[11,14-18]. These dynamics are likely driven by an initial expansion of plasmablasts which produce large amounts of antibody but die off quickly followed by a slower decay of antibody titres (the half-life of IgG is approximately three weeks) which then transitions into a steady state level of antibody produced by long-lived plasma cells[19]. However, it is currently unknown, if the magnitude of the initial expansion of plasmablast and the associated antibody titres are correlated with the steady state level of serum antibody produced by long-lived plasma cells. This is an important question since steady state antibody levels may provide superior protection from re-infection[20,21].

Specifically, there is currently a paucity of information on the kinetics of antibody decay among health care workers (HCW). It is suspected that SARS-CoV-2 infections among HCW are usually asymptomatic or mildly symptomatic and frequently associated with either underreporting of symptoms or heterogenous PCR and/or serologic diagnostics leading to most of them going undetected or unrecognized[22]. A large cohort study of HCWs in the greater New York City (NYC) area showed a seroprevalence of SARS-CoV-2 antibodies of 13.7%[23]. Our own data of anti N antibody screening among HCW at a New York City public hospital in the Bronx following the first "surge" of COVID-19 in May 2020, found that SARS-CoV-2 seroprevalence was at 27%[24]. Understanding the longitudinal kinetics of SARS-CoV-2 antibody response and the effectiveness of commercial antibody measurement assays is crucial to correctly determine infection rates, sero-prevalence and true sero-reversion rates in both

infected and vaccinated individuals – and to better understand protection associated with sero-positivity.

In this study, we aimed to investigate the longitudinal humoral responses to viral N and the spike and to evaluate their correlation to clinical symptoms and baseline characteristics in our HCW study cohort. Importantly, having access to samples during the initial antibody peak and several months out, we also aimed to determine if initial high antibody levels correlated with high antibody titers at steady state.

Methods

Study setting and population

The study is a prospective cross-sectional cohort study done in two phases after receiving Institutional Review Board approval (IRB # 20-009). The Phase 1 was conducted in May/June, 2020 and the Phase 2 was completed August/September 2020. The cohort included HCWs who worked at the New York City Public Hospital in the South Bronx and were willing to participate in both phases of the study. Information about the study was disseminated among health care workers via intranet informative bulletins, research staff approaching on duty staff and handing our study flyers and introducing the study in multiple department meetings. During Phase 2, the HCWs that had participated for Phase 1 were called individually to schedule an appointment with research staff for blood-work and survey completion.

In the Phase 1 of the study, after informed consent, participants underwent qualitative serology testing (Abbott Architect SARS-CoV-2 IgG Assay, Abbott Park, IL 60064 USA)[25] and a nasopharyngeal swab for SARS-CoV-2 (Bio-Reference Laboratories, Inc., Elmwood Park, NJ, USA). They also completed an initial online survey on demographics, symptoms of COVID-

19 including duration, and healthcare/community exposure. An extra sample was collected and stored at -80°C for subsequent analysis. These samples were processed using a quantitative enzyme-linked immunosorbent assay (ELISA) that correlates well with virus neutralization, developed by Mount Sinai Health System (MSH ELISA)[26,27], to detect RBD and full-length spike (S) reactive antibodies; and in Phase 2 for follow up serology testing was also performed with Abbott and MSH ELISA.

Antibody assays

The Abbott Architect assay uses a qualitative chemiluminescent microparticle immunoassay technology targeting the N antigen of the virus with a reported sensitivity of 100% (CI 95.8–100%) and specificity of 99.6% (CI 99–99.9%)[25]. The MSH ELISA consists of an initial ELISA using serum or plasma to detect specific IgG against the RBD of SARS-CoV-2 at a single dilution, followed by quantitative titrations of presumptive positives in a confirmatory ELISA against full length SARS-CoV-2 spike protein (S)[28]. The positive result from the spike ELISA is reported as antibody at a titre of 1:80 or higher. Test performance assessment revealed that PCR+ samples were 94 % positive and all negative samples returned a negative result for 100% negative agreement[29].

Survey

The online survey was accessed by a unique identification number assigned to each participant, blinded to the research team to ensure confidentiality. The survey was designed for the purpose of this study to capture demographics and current medical history, with emphasis on COVID-19 infection exposure, symptomology, duration of symptoms and persistence of symptoms from exposure prior to Phase 1 (period prior to May 2020). Survey was piloted amount staff prior to use in this study. The survey requested information on age, race/ethnicity,

comorbidities, domestic/international travel and healthcare and community exposure details during and prior to both phases. The first phase collected information about symptoms of COVID-19 including their timing and duration in the preceding weeks of the blood draw[24]. The Phase 2 survey requested information on new comorbidities, persistent COVID-19 symptoms (cough, shortness of breath, anosmia, ageusia, myalgia, nausea, and/or diarrhea), interim testing via antibody and/or reverse transcription polymerase chain reaction (RT -PCR) (if present) and their result (positive/negative), presence of positive SARS-CoV-2 PCR results in the preceding months, interim domestic/international travel and continued use of personal protective equipment (PPE). The risk of exposure in the healthcare setting and community exposure was determined based on CDC guidelines[30].

Statistical analysis

Convenience sampling design was adapted to recruit participants with a goal of 500 participants. Descriptive statistics were used to summarize the baseline characteristics of the cohort and key study outcome variables. Categorical variables were compared by the Chi–squared test, while continuous variables were compared by a Student's t-test. The spike antibody titres were described as geometric means. Correlations were calculated using standard Pearson and Spearmen correlation. Multiple linear regression was applied to determine the predictors of log10 rate of decay from Phase 1 to Phase 2 of anti-spike antibodies. A p-value of <0.05 was considered significant. All statistical analyses were performed using SPSS version 27 (IBM, USA).

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Results

For Phase 1 of our study, 500 healthcare workers underwent both PCR and serology testing. Of these, 137 were positive by for anti-N antibody (Abbott) and 142 were positive by the MSH ELISA. For the second phase 178 participants from the initial cohort consented and underwent evaluation with PCR, antibody assays (Abbott and MSH ELISA) and completed the online follow up survey. The follow-up occurred 133±21 days between Phase 1 and Phase 2. The details of patient enrolment are described in **Figure 1**. While 46 of the 178 tested subjects remained positive for the anti-N antibody (Abbott), 70 were positive by the MSH ELISA in the second phase. Anti -spike titres of the 5 subjects in the first phase were close to the cut off for positivity. Twenty-two subjects who were negative for anti-N antibody in Phase 2 had positive titres of anti-RBD and anti-spike antibodies, though lower than their Phase 1 levels. Among the subjects who participated in the Phase 1 and Phase 2 study, 68 were positive in both phases by the MSH ELISA, 110 were negative in both phases and 2 were positive only in Phase 2 with previously negative results in Phase 1.

The baseline characteristics of study participants who were positive by MSH ELISA in both phases (n=68) and those who were negative in both phases (n=108) are shown in **Table 1**. The mean age of the participants was 44.7±12.4 years, and 63.1% were female. Overall, 30.7% of the HCWs were Latinx, 29.5% were Asian, 16.5% were Black and 17.6% were White. COVID-19 related symptoms were present in 83.8% (57) of the subjects who were positive in both phases, while only 42.6% (46) of the subgroup who had negative antibodies in both phases admitted to symptoms prior to Phase 1. The duration of symptoms prior to Phase 1 was longer among the symptomatic positive group (48.3% for >14 days) in comparison to symptomatic negative group (17.8% for >14 days). The mean duration of symptoms to Phase 1 testing in the

symptomatic positive sub cohort was 47.9 ±16.0 days. Persisting symptoms of COVID-19 were reported in 19 (27.9%) subjects from the cohort with positive antibodies in both phases.

Clinical characteristics and seropositivity to spike protein in both phases

Table 2 describes the characteristics of the symptomatic and asymptomatic subjects who were positive for anti-spike antibody in both phases. Baseline characteristics were comparable between the groups and no difference either in the healthcare or community exposure or in the location of work (ED/Inpatient/intensive care unit, OR etc.) between the two groups was observed. Titres of anti-spike antibodies (geometric mean area under the curve (AUC)) were higher in symptomatic subjects than in asymptomatic positive subjects (6754 AUC vs. 5803 AUC) in Phase 1. However, in the Phase 2 analysis we observed marginally higher titres in the asymptomatic subgroup compared to the symptomatic subgroup (2383 AUC vs. 2198 AUC). Figure 2 illustrates the symptomatic and asymptomatic antibody levels of anti-spike antibodies. The rate of decay was higher in the symptomatic subgroup (geometric mean 32.96 per day) compared to the asymptomatic (geometric mean 23.42 per day) suggesting delayed antibody/kinetics in the asymptomatic cohort.

Phase 1 anti-spike antibody titre and clinical correlations

A Pearson's product and Spearman's rank-order correlation was run to assess the relationship between cohort characteristics including age, gender, comorbidities, number of symptoms of COVID-19, healthcare exposure and Phase 1 anti-spike titres in our cohort (**Figure 3**). One hundred-forty-three subjects with a positive test in Phase 1 were included in the analysis. Scatter plot analysis showed a monotonic relationship between the variables. A statistically significant weak positive correlation was observed between age and Phase 1 anti-spike antibody titres ($R=0.269$, $p<0.005$). Moderate positive correlation was present between

presence of fever (R=0.319, p<0.005), number of symptoms (R=0.310, p<0.005) and days of symptoms (R=0.434, p<0.005) and anti-spike antibody titre; and weak positive correlation was observed with upper respiratory symptoms (R=0.278, p<0.005) and gastrointestinal (GI) symptoms (R=0.204, p<0.05) with anti-spike antibody titres.

Correlation of rate of decay of anti-spike antibody titres from Phase 1 to Phase 2 and clinical characteristics

Results of Pearson correlation to assess the relationship between cohort characteristics including Phase 1 anti-spike antibody titres, age, gender, comorbidities, symptoms of COVID-19, number of symptoms of COVID-19, healthcare exposure and decay of anti-spike titres between the two phases in our cohort is shown in **Figure 4.** A strong positive statistically significant correlation was observed between Phase 1 titers and decay of anti-spike antibody titres between the two phases (R=0.898, p<0.000). Medium positive correlation was observed between presence of fever (R=0.428, p<0.001), GI symptoms (R=0.340, p<0.011), number of symptoms (R=0.357, p<0.007), duration of symptoms (R=0.469, p<0.000) with decay of anti-spike antibody titres between the two phases respectively.

A pairwise comparison was performed between rate of decay of anti-spike antibody titres and patient characteristics (**Figure 5**). Rate of decay by gender was comparable (male; 30.73 AUC/day vs. female;34.68 AUC/day, p=0.413). Asian (86.0 AUC/day) race showed higher rate of decay compared with White (7.2 AUC/day) and Black (19.61 AUC/day) individuals; while Latinx (47.28 AUC/day) race had higher rate of decay compared with White (7.2 AUC/day) individuals. Subjects with fever had a higher rate of decay than those who did not report fever (53.08 AUC/day vs.16.14 AUC/day, p=0.002). Similarly subjects with GI

symptoms had a higher rate of decay than those without (55.81 AUC/day vs.21.94 AUC/day, p=0.019). Subjects with symptoms restricted to less than seven days demonstrated a lower decay rate compared with symptomatic subjects over 7-14 days (13.60 AUC/day vs. 36.12 AUC/day, p=0.046) and when compared with symptomatic subjects with more than 14 days (13.60 AUC/day vs. 59.72 AUC/day, p=0.001). This finding was statistically significant. No difference was found when degree of exposure (High/Moderate: 28.18 AUC/day vs. Mild: 34.78 AUC/day, p=0.395) or job role (physician: 29.57 AUC/day vs. nurse: 53.59 AUC/day vs. Other: 26.83 AUC/day; p=0.361) was compared to rate of decay.

Predictors of rate of decay from Phase 1 to Phase 2 of anti-spike antibodies

Multiple linear regression analysis to predict the rate of decay with respect to age, Bacillus Calmette Guerin vaccination, number of symptoms, and Phase 1 (log10) anti-spike antibody titres is shown in **Table 3**. On the basis of a linear regression model that included the participants age, history of BCG vaccination, total number of COVID-19 symptoms and the Phase 1 concentration of log 10 spike antibody titres, the estimated change (decay) was 23.6 AUC/day when age was centred at median (42.6 years), there was positive history of BCG vaccination, the total number of COVID-19 symptoms were centred at a median of 4, and the geometric mean of the $\log_{10}$ spike antibody titre was 3.78.

Discussion

With the COVID-19 pandemic showing no signs of abating, healthcare workers at the epicentre are at risk of infection due to occupational exposure as well as community exposure. Sero-surveillance is the foundation for determining the scale and rate of exposures. With a multitude of serological assays getting emergency use approval from FDA, interpretation of the results of these assays and their clinical significance remains

challenging. It is critical to understand the timing of the antibody response for acute interpretation. Confidence in analytical specificity of the assay is a critical requirement in measurement of the specific antibody responses. Recent studies have confirmed that anti spike titres especially anti-RBD titres can serve as surrogates for virus neutralization[31,32]. The Abbott SARS-CoV-2 IgG assay that targets antibodies to the nucleoprotein has a reported specificity and sensitivity of greater than 99% at 14 days or more following symptom onset and these measurements are not indicative or correlated to virus neutralization titres[33]. In comparison, the MSH ELISA targets the full-length S protein including RBD, a major target for neutralizing antibodies and has demonstrated excellent correlation to virus neutralization[11,26]. Longitudinal measurements of antibody levels have revealed that anti-N and anti S IgG antibodies continue to increase until the third week post symptoms and an approach that combines the detection of both of these antibodies would precisely detect almost 100% of all infectious exposures[34]. In our study, the mean number of days after symptoms to testing in Phase 1 was 47 days suggesting a higher likelihood of accuracy of the utilized assay.

Longitudinal blood sampling among HCWs working at a public hospital in the epicentre of the pandemic in NYC allowed for analysis of kinetics of anti-S and anti-N antibody responses. At two months after the first surge of infections, anti-N antibodies were detected in 27% and anti-S antibodies in 28% of participating HCWs. After an interval of four months, it is not surprising to note that among the participants who returned, 26% remained positive for anti N antibodies, while 31% of the previously anti-N antibody positive subjects tested negative in phase 2. On the other hand, a similar analysis of the anti-S antibodies levels, confirmed that all the previously positive retested subjects continued to remain positive, albeit with lower titres. That being said, we acknowledge that the decline of N antibodies in

our cohort could be due to the Abbott assay being less sensitive to describe the dynamics of N antibodies over time compared to other assays, like Roche, Siemens and Diasorin. Muecksch et al. demonstrated in their longitudinal analysis of clinical serology assay performance among COVID-19 convalescents, that there is a difference in diagnostic performance among various serologic assays[32].

COVID-19 related symptoms were significantly associated with positive anti-spike antibodies in both phases, with a similar association with longer duration (>14 days) of symptoms. Previous studies have demonstrated a lower level of IgG response among patients without symptoms or with mild symptoms compared to those with severe and critical disease[35,36]. A comparison of symptomatic versus asymptomatic subjects who tested positive for anti-spike antibodies in both phases, confirmed that the rate of decay of anti-spike antibody titres were faster in the symptomatic cohort than the asymptomatic subjects, which was seen also in the anti-N antibody kinetics. However, we observed a faster decay in this group with a lower titre of anti-spike antibodies in Phase 2 compared to the asymptomatic cohort (though the difference was not statistically significant). This could additionally be supported by the finding of fever and GI symptoms contributing to faster decay. Similar results of decreasing neutralizing antibody titre in symptomatic than asymptomatic patients were observed by Choe *et al.*[37]

Positive correlations for age, presence of fever, upper respiratory symptoms, GI symptoms, total number and duration of symptoms was observed with increased levels of anti-spike titres at Phase 1. Similar results of neutralizing antibody titres were also observed by Boonyaratanakornkit *et al.* wherein they showed higher levels of neutralizing antibody titres were significantly associated with male gender, older adults, higher disease severity and shorter interval from recovery[38]. Based on a linear regression model with age centred at median (42.6

years), positive history of BCG vaccination, the total number of COVID-19 symptoms centred at a median of 4, and the geometric mean of the log10 anti-spike antibody titre at 3.78, we observed that the rate of decay of these antibody titres was 23.6 AUC/day. Evaluation of other characteristics with rate of decay between Phase 1 and Phase 2 showed a faster reduction in titres in Asian participants and in those with fever and GI symptoms. A slower decrease was noted among patients with shorter duration (<7 days) of symptoms, with no other significant correlation noted with any other baseline demographics or clinical characteristics.

As described above, higher antibody titers are associated with a larger number of symptoms, longer duration of symptoms and – as described by others as well – disease severity in general. We also found that higher initial antibody titers were associated with faster antibody decay during the two time points. Initial antibody responses are driven by short lived plasmablasts, which decay after a few days after producing massive amounts of antibody. IgG has a relatively long half-life of approximately three weeks, but decay is inevitable since the plasmablasts initially producing it disappear. Usually, titers then drop until they reach relatively stable levels of antibody which are maintained by long-lived plasma cells in the bone marrow[19]. The two time points described in this study represent the initial peak response and likely the stable level after the initial decay. We found that individuals with higher initial titers had a faster decay rate during the observation period meaning the difference between peak and stable, long-lived antibody levels were larger. This indicates that there is likely no direct correlation between the magnitude of the initial expansion of plasmablasts and the number of long-lived plasma cells that migrate to the bone marrow. It is critical to recognize that steady state antibody titers are similar between the symptomatic and asymptomatic subgroups, suggesting that mid-term humoral protection might be similar after infection regardless of disease severity.

Our study has the following limitations: First being a single center study with a small convenience sampling that included a smaller number of participants in Phase 2 of the study. Following the pandemic, the HCWs who volunteered from around the country were transferred back and lost to follow-up, which did decrease the overall sample size, but the rates of positive and negative results remained proportional. Second, the likelihood of a recall bias in the participant's responses on the online survey may exist. Lastly, as a cross-sectional seroprevalence study the findings can underestimate rates of prior infections based on timing of the testing given that antibodies are only transiently detectable following infection.

In conclusion, findings from this study are similar to other studies that have reported that higher magnitude of anti-spike titres may correlate with protection against reinfection, in spite of the observed decay in the antibody levels[20,21]. Nevertheless, further studies to evaluate the longevity of immunity, especially in context of widespread administration of spike-based vaccine among HCWs would be important in predicting herd immunity to COVID-19 infections.

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Contributions: V.M., M.A.S. and U.V. designed the study. J.M.C., M.A.S., B.Y., V.P.G. analysed the data. E.V. and M.G. assisted with participant follow-up and coordination with the assistance of A.P., U.V., V.M., and M.A.S. The Mount Sinai Health System team, J.M.C. and F.K., performed the measurements for anti-Spike and Anti-RBD antibodies. V.M., U.V. and A.P. were responsible for the clinical care of the research participants and supervised the day-to-day operation and coordination of the study by M.K., V.D., M.A.S., B.Y., V.P.G., M.G., E.V., and M.G. V.M. and F.K wrote the manuscript and are the guarantors of this work and have full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, with the assistance of J.C.Q., M.A.S., B.Y., V.P.G., and M.G. All authors critically revised the draft and approved the final manuscript.

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Competing Interests: F.K. is listed as a co-inventor on a patent application filed by The Icahn School of Medicine at Mount Sinai relating to SARS-CoV-2 serological assays and NDV-based SARS-CoV-2 vaccines. Mount Sinai has spun out a company, Kantaro, to market serological tests for SARS-CoV-2. F.K. has consulted for Merck and Pfizer (before 2020), and is currently consulting for

Seqirus and Avimex. F.K.'s Krammer laboratory is also collaborating with Pfizer on animal models of SARS-CoV-2.

All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Patient and public involvement: Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication: Not required

Ethics approval: The study protocol was approved by the Institutional Review Board approval (IRB # 20-009, Lincoln Medical Center, Office of the Institutional Review Board approved as per 45 CFR 46 & 21 CFR50,56 under a full board committee and gave its approval on 4/28/2020). All participants provided written informed consent for the use of their data.

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Table 1: Broad characteristics among health care workers assessed for antibody reactivity to spike SARS-CoV-2 protein in Phase 1 and Phase 2

	Overall †	Spike ELISA (AUC) positive in both phases	Negative Reactivity to spike (AUC) in both phases	p value
	N=176	n=68	n=108	
Age, years	44.7+12.4	42.9+11.9	45.8+12.7	0.099
Female, Gender	111 (63.1%)	40 (58.8%)	71 (65.7%)	0.467
Race				0.666
Latinx	54 (30.7%)	21 (30.9%)	33 (30.6%)	
Asian	52 (29.5%)	18 (26.5%)	34 (31.5%)	
Black	29 (16.5%)	15 (22.1%)	14 (13.0%)	
White	31 (17.6%)	10 (14.7%)	21 (19.4%)	
Other	10 (5.7%)	4 (5.9%)	6 (5.9%)	
Comorbidities	54 (30.7%)	25 (36.8%)	29 (26.9%)	0.214
BCG vaccine received in childhood	87 (49.4%)	35 (51.5%)	52 (48.1%)	0.902
COVID-19 related symptoms prior to Phase 1	103 (58.5%)	57 (83.8%)	46 (42.6%)	<.001
Duration of symptoms				<.001
<7 days	48 (46.6%)	18 (31.0%)	30 (66.7%)	
7-14 days	19 (18.4%)	12 (20.7%)	7 (15.6%)	
>14 days	36 (35.0%)	28 (48.3%)	8 (17.8%)	
Time from symptom to positive result, days	45.7+19.9	47.9+16.0	42.9+24.1	0.062
RT-PCR positive result for SARS-CoV-2 prior to Phase 1	51 (29.0%)	49 (72.1%)	2 (1.9%)	<.001
RT-PCR positive result for SARS-CoV-2 during Phase 1	14 (8.0%)	13 (19.1%)	1 (0.9%)	<.001
Persisting symptoms from COVID-19	25 (14.2%)	19 (27.9%)	6 (5.6%)	<.001
Nature of work				0.306
Physicians	81 (46.0%)	29 (42.6%)	52 (51.5%)	
Nurses	29 (16.5%)	15 (22.1%)	14 (13.0%)	
Others	64 (36.4%)	24 (35.3%)	40 (39.6%)	
Hospital areas worked in:				
Emergency department/Inpatient units	118 (67.0%)	50 (73.5%)	68 (63.0%)	0.141
Ambulatory care/Clinics	72 (40.9%)	27 (39.7%)	45 (41.7%)	0.631
Administration/Non-clinical care areas	24 (13.6%)	9 (13.2%)	15 (13.9%)	0.867
Community exposure	47 (26.7%)	19 (27.9%)	28 (25.9%)	0.591

Household exposure	39 (22.2%)	17 (25.0%)	22 (20.4%)	0.343
PPE use at work	173 (98.3%)	67 (98.5%)	106 (98.1%)	0.226
Use of facemask outside of the hospital	158 (89.8%)	58 (85.3%)	100 (92.6%)	0.062

Continuous variables are expressed as mean (SD), categorical variables as n (%).
BCG, Bacillus Calmette–Guérin vaccine; PPE, personal protective equipment; RT-PCR, reverse transcription polymerase chain reaction.
† Demographic data is missing for 2 participants from the overall cohort.

Table 2: Broad characteristics among health care workers with positive antibody reactivity to SARS-CoV-2 spike in both phases

	Overall	Asymptomatic for SARS-CoV-2 infection	Symptomatic for SARS-CoV-2 infection	*p value*
	n=68	n=11	n=57	
Age, Mean (±SD)	42.9 (±1.45)	44.5 (±3.8)	42.6 (±1.6)	0.557
Female, n (%)	40 (58.8%)	6 (54.5%)	34 (40.4%)	0.502
Race				0.753
Latinx	21 (30.9%)	3 (27.3%)	18 (31.6%)	
Asian	18 (26.5%)	3 (27.3%)	18 (26.3%)	
Black	15 (22.1%)	3 (27.3%)	12 (21.1%)	
White	10 (14.7%)	2 (18.2%)	8 (14.0%)	
Other	4 (5.8%)	0 (0%)	4 (7.0%)	
Comorbidities				
Hypertension	13 (19.1%)	2 (18.2%)	11 (19.3%)	0.650
Diabetes	6 (8.8%)	0 (0%)	6 (10.5%)	0.332
COPD and asthma	13 (19.1%)	1 (9.1%)	12 (21.1%)	0.326
Number of symptoms, median (IQR)	-	-	4.0 (2.0-5.0)	
Length of symptoms				
<7 days	-	-	19 (33.3%)	
7-14 days	-	-	12 (21.1%)	
>14 days	-	-	26 (45.6%)	
Degree of HCW exposure				0.492
High and Moderate	16 (23.5%)	2 (18.2%)	14 (24.6%)	
Minor	52 (76.5%)	9 (81.8%)	43 (75.4%)	
Community exposure	19 (27.9%)	3 (27.3%)	16 (28.1%)	0.635
Household exposure	17 (25.4%)	3 (27.3%)	14 (24.6%)	0.557
Use of facemask outside of hospital	58 (85.3%)	9 (81.8%)	49 (86.0%)	0.722

Principal means of transportation				0.663
Public	33 (48.5%)	6 (54.5%)	27 (47.7%)	
Private	35 (51.5%)	5 (45.5%)	30 (52.6%)	
Nature of work				0.502
Physician	29 (42.6%)	4 (36.4%)	25 (43.9%)	
Nurse	15 (22.1%)	2 (18.2%)	13 (22.8%)	
Other	24 (35.3%)	5 (45.5%)	19 (33.3%)	
Hospital areas work in:				0.288
Emergency department/inpatient units	32 (47.1%)	6 (54.5%)	26 (45.6%)	
Ambulatory care/clinics	9 (13.2%)	2 (18.2%)	7 (12.3%)	
Impatient and outpatient setting	18 (26.5%)	3 (27.3%)	15 (26.3%)	
Administration/nonclinical care areas	9 (13.2%)	0 (0%)	9 (15.8%)	
Anti-spike reactivity (AUC)				
Reactivity in phase 1, G-Mean (IQR)	6590 (5165-8410)	5803 (2825-11920)	6754 (5177-8812)	0.647
Days from symptoms to first test, Mean (±SD)	-	-	47.7 (±1.9)	
Reactivity in phase 2, G-Mean (IQR)	2226 (1824-2718)	2382 (1494-3797)	2198 (1753-2755)	0.980
Days from symptoms to second test			174.5 (±4.1)	
Rate of decay, G-Mean (IQR)	31.14 (22.11-43.87)	23.42 (8.45-64.93)	32.96 (22.73-47.82)	0.382

Continuous variables are expressed as mean (SD) or interquartile range (IQR), categorical variables as n (%).

AUC, area under the curve; COPD, Chronic obstructive pulmonary disease; HCW, health care worker

Table 3: Multiple linear regression analysis of rate of decay for anti-spike antibodies between Phase 1 and Phase 2

Rate of decay (log10)	*B*	95.0% CI for B		*SE B*	ß	R^2	▲R^2
		LL	UL				
Model						0.83	0.82
Constant	-3.203**	-3.647	-2.759	.222			
Age (per 10-year change)	.014	-.005	.007	.002	.030		
BCG vaccination	.131**	.030	.310	.046	.121		
Number of symptoms	.013	-.029	.060	.012	.050		
ELISA reactivity (Log10)	1.159**	1.050	1.419	.059	.916		

*B: Un*standardized regression coefficient; CI: confidence interval; LL: lower limit; UL: upper limit; SE B: standard error of the coefficient; ß: standardized coefficient; R^2: coefficient of determination; ▲R^2: adjusted R^2.

***P<0.*05

BCG, Bacillus Calmette–Guérin vaccine

550

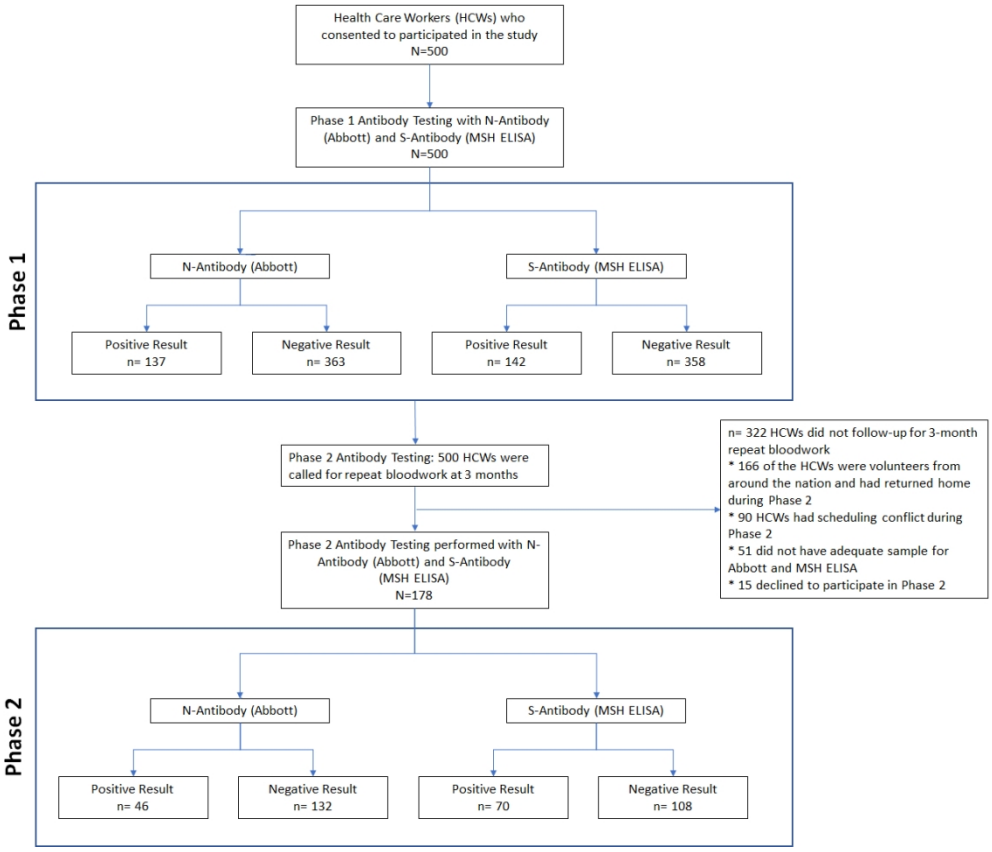
Figure 1: Flow Chart of patient enrollment, follow up and analysis

Figure 2: Antibody levels from Phase 1 in specimens obtained early during the pandemic (May 2020) and Phase 2 in follow up visit (August-October 2020) are shown for symptomatic and asymptomatic participants.

Figure 3: Simple correlation analysis of HCW with positive reactivity for anti- spike antibody in Phase 1 with baseline characteristics and symptoms

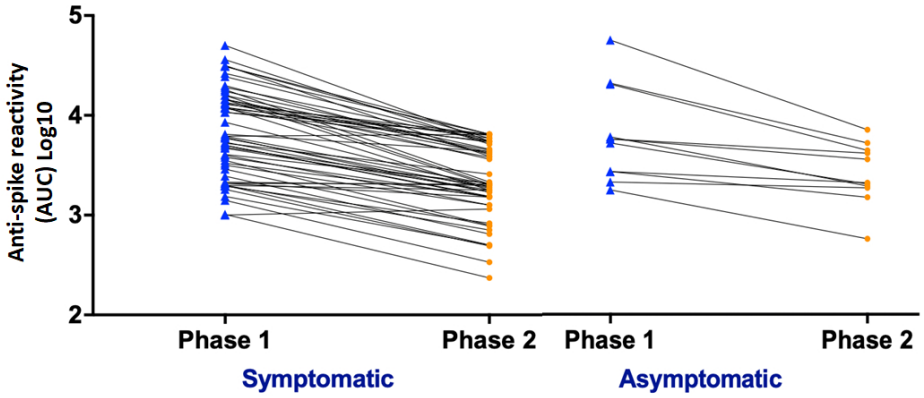
Figure 4: Simple correlation analysis of rate of decay of anti-spike antibodies between both phases with baseline characteristics and symptoms

Figure 5: Paired comparison between rate of decay of anti-spike antibody titres and patient characteristics



Flow Chart of patient enrollment, follow up and analysis

340x286mm (96 x 96 DPI)



Antibody levels from Phase 1 in specimens obtained early during the pandemic (May 2020) and Phase 2 in follow up visit (August-October 2020) are shown for symptomatic and asymptomatic participants.

295x129mm (96 x 96 DPI)

		ELISA reactivity in Phase 1 (Log10)	Age	Gender	Comorbidities	BCG vaccine	Fever	URS	GI symptoms	Number of symptoms	Days of symptoms	Degree of HCW exposure
ELISA reactivity in Phase 1 (Log10)	R	1										
	Sig.	-										
Age	R	0.269**	1									
	Sig.	0.001	-									
Gender	R	0.106	0.050	1								
	Sig.	0.209	0.552	-								
Comorbidities	R	0.130	0.468**	0.045	1							
	Sig.	0.123	0.000	0.596	-							
BCG vaccine	R	0.046	0.014	-0.129	0.084	1						
	Sig.	0.585	0.869	0.125	0.317	-						
Fever	R	0.319**	0.024	-0.089	0.018	0.060	1					
	Sig.	0.000	0.777	0.292	0.828	0.479	-					
URS	R	0.278**	0.039	0.059	0.069	-0.214*	0.464**	1				
	Sig.	0.001	0.645	0.486	0.413	0.010	0.000	-				
GI symptoms	R	0.204*	0.030	0.068	0.029	0.024	0.344**	0.291**	1			
	Sig.	0.015	0.723	0.423	0.733	0.775	0.000	0.000	-			
Number of symptoms	R	0.310**	-0.015	0.070	0.026	0.019	0.708**	0.665**	0.630**	1		
	Sig.	0.000	0.856	0.405	0.762	0.825	0.000	0.000	0.000	-		
Days of symptoms	R	0.434**	0.036	0.019	0.121	-0.038	0.422**	0.605**	0.347**	0.681**	1	
	Sig.	0.000	0.671	0.817	0.151	0.656	0.000	0.000	0.000	0.000	-	
Degree of HCW exposure	R	-0.145	-0.215**	-0.217**	-0.177*	0.017	0.017	0.034	0.000	0.039	-0.085	1
	Sig.	0.083	0.010	0.009	0.034	0.838	0.844	0.690	0.998	0.640	0.314	-

BCG, Bacillus Calmette–Guérin; URS, upper respiratory symptoms; GI, Gastrointestinal symptoms (nausea, vomit and diarrhoea); HCW, health care worker.
**. Correlation is significant at the 0.01 level., *. Correlation is significant at the 0.05 level.

Simple correlation analysis of HCW with positive reactivity for anti- spike antibody in Phase 1 with baseline characteristics and symptoms.

767x285mm (96 x 96 DPI)

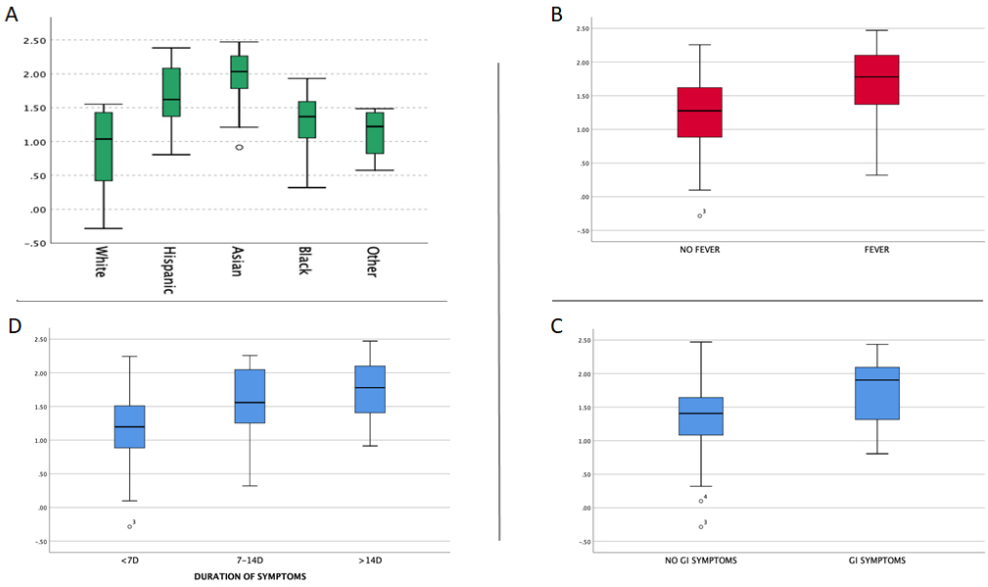
		Rate of decay (Log10)	ELISA reactivity (Log10)	Age	Gender	Comorbidity	Fever	GI symptoms	Number of symptoms	Duration of symptoms	Degree of HCW exposure
Rate of decay (Log10)	R	1									
	sig.	-									
ELISA reactivity (Log10)	R	0.898**	1								
	sig.	0	-								
Age	R	0.176	0.182	1							
	sig.	0.198	0.184	-							
Gender	R	0.044	0.094	0.001	1						
	sig.	0.751	0.493	0.994	-						
Comorbidity	R	0.193	0.156	0.514**	-0.092	1					
	sig.	0.158	0.257	0.000	0.502	-					
Fever	R	0.428**	0.463**	-0.090	-0.090	-0.122	1				
	sig.	0.001	0.000	0.515	0.512	0.374	-				
GI symptoms	R	0.340*	0.289*	-0.020	-0.072	-0.163	0.269*	1			
	sig.	0.011	0.033	0.885	0.603	0.234	0.047	-			
Number of symptoms	R	0.357**	0.360**	-0.256	0.008	-0.193	0.640**	0.555**	1		
	sig.	0.007	0.007	0.059	0.954	0.158	0.000	0.000	-		
Duration of symptoms	R	0.469**	0.469**	-0.049	0.123	0.142	0.204	0.207	0.430**	1	
	sig.	0.000	0.000	0.723	0.370	0.300	0.136	0.129	0.001	-	
Degree of HCW exposure	R	0.067	0.106	0.154	0.266*	0.202	-0.136	-0.075	-0.184	0.133	1
	sig.	0.625	0.439	0.261	0.050	0.140	0.321	0.586	0.180	0.334	-

BCG: Bacillus Calmette–Guérin; GI: Gastrointestinal symptoms (nausea, vomit and diarrhea); HCW: health care worker.

**. Correlation is significant at the 0.01 level, *. Correlation is significant at the 0.05 level

Simple correlation analysis of rate of decay of anti-spike antibodies between both phases with baseline characteristics and symptoms.

686x269mm (96 x 96 DPI)



Paired comparison between rate of decay of anti-spike antibody titres and patient characteristics.

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Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	#3	State specific objectives, including any prespecified hypotheses	7
Methods			

Study design	#4	Present key elements of study design early in the paper	7
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	7
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	7
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	8
Bias	#9	Describe any efforts to address potential sources of bias	16
Study size	#10	Explain how the study size was arrived at	9
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	7-9
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	9
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	9
Statistical methods	#12c	Explain how missing data were addressed	9
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	9
Statistical methods	#12e	Describe any sensitivity analyses	9

Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	9
Participants	#13b	Give reasons for non-participation at each stage	30
Participants	#13c	Consider use of a flow diagram	
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	9,23-24
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	**9,23-26**
9,23-26			
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	**4, 10**
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	**11**
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-11
Main results	#16b	Report category boundaries when continuous variables were categorized	10
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	**11**

Other analyses	#17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11-13
Discussion			
Key results	#18	Summarise key results with reference to study objectives	13-14
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	16
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	15-16
Generalisability	#21	Discuss the generalisability (external validity) of the study results	16-17
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

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Longitudinal Humoral Antibody Response to SARS-CoV-2 Infection among Health Care Workers in a New York City Hospital

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Keywords:	COVID-19, INFECTIOUS DISEASES, IMMUNOLOGY

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Longitudinal Humoral Antibody Response to SARS-CoV-2 Infection among Health Care Workers in a New York City Hospital

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Abstract

Objective

Dynamics of humoral immune responses to SARS-CoV-2 antigens following infection suggests an initial decay of antibody followed by subsequent stabilization. We aim to understand the longitudinal humoral responses to SARS-CoV-2 nucleocapsid (N) protein and spike (S) protein and to evaluate their correlation to clinical symptoms among healthcare workers (HCW).

Design

A prospective longitudinal study.

Setting

This study was conducted in New York City Public Hospital in the South Bronx, New York.

Participants

HCWs participated in Phase 1 (N=500) and were followed up 4 months later in Phase 2 (N=178) of the study. They underwent SARS-CoV-2 PCR and serology testing for N and S protein antibodies, in addition to completion of an online survey in both phases. Analysis was performed on the 178 participants that participated in both phases of the study.

Primary outcome measure

Evaluate longitudinal humoral responses to viral N (qualitative serology testing) and Spike protein (quantitative MSH-ELISA to detect Receptor binding domain and full-length S reactive antibodies) by measuring rate of decay.

Results

Anti-N antibody positivity was 27% and anti-S positivity was 28% in Phase 1. In Phase 1 anti-S titers were higher in symptomatic (6754 [5177-8812]) than in asymptomatic positive subjects (5803 [2825-11920]). Marginally higher titers (2382 [1494-3797]) were seen in asymptomatic compared to the symptomatic positive subgroup (2198 [1753-2755]) in Phase

2. A positive correlation was noted between age (R=0.269, p<0.01), number (R=0.310, p<0.01) and duration of symptoms (R=0.434, p<0.01), and Phase 1 anti-S antibody titer. A strong correlation (R=0.898, p<0.001). was observed between Phase 1 titers and decay of anti-S antibody titers between the two phases. Significant correlation with rate of decay was also noted with fever (R=0.428, p<0.001), GI symptoms (R=0.340, p<0.05), and total number (R=0.357, p<0.01) and duration of COVID-19 symptoms (R=0.469, p<0.001).

Conclusions

Higher initial anti-S antibody titers were associated with larger number and longer duration of symptoms as well as a faster decay between the two time points.

Strengths and limitations of this study

- The strength of our study is the longitudinal design with serial sampling to determine humoral response to SARS-CoV-2 infection from consenting Health Care Workers during the pandemic.
- This study collected serial detailed characteristics of symptomatic and asymptomatic Health Care Workers to correlate with durability and decay of humoral response.
- This study is limited by representation of only a single institution's data and the possibility of recall bias to the responses on the online survey.
- Our cohort for Phase 2 was smaller than Phase 1, due to discontinuation of volunteer healthcare workers from the surge period.

Introduction

In light of the unprecedented Coronavirus Disease 2019 (COVID-19) pandemic, understanding the role of the immune system in countering the viral infection is critical not just to design effective antiviral strategies but also to aid us in taking appropriate public health decisions. The early publication of the viral genome led to a rapid development of many nucleic acid based diagnostic assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections. While nucleic acid-based tests are widely employed in the diagnosis of acute (current) SARS-CoV-2 infections, they are often limited in their clinical utility in identifying past infections or assess the level of immunity to SARS-CoV-2 within the communities. Evaluation of antibody responses is the other well-known modality used in a clinical setting that can detect both current, and past infections and is the preferred approach for surveillance to determine the true prevalence of infections. The currently available serological assays for SARS-CoV-2 target either the viral nucleoprotein (N) or the spike surface protein (S) antigens. The S-protein, which contains the receptor binding domain (RBD), binds to host cells via the angiotensin converting-enzyme-2 (ACE2) receptor, followed by membrane fusion[1,2]. The spike is the target of most neutralizing antibodies[3-5], while the N plays an important role in transcription enhancement and viral assembly[6]. Studies have demonstrated that antibodies against the N and S appeared around the same time - between day 8 and day 14 after the onset of symptoms with antibodies to the N being more sensitive than anti S antibodies for detecting early infection[7]. Neutralizing antibodies confer protective immunity and can be detected in most infected individuals 10-15 days following the onset of COVID-19 symptoms and remain elevated following initial viral clearance[8-12]. There is compelling evidence to suggest that serological assays for anti-S antibodies predict neutralizing activity, in contrast to N based assays[11,13].

The detailed characterization of the dynamics of humoral immune responses to the SARS-CoV-2 viral antigens following infection is still ongoing and early evidences suggest an initial decay of antibody followed by stabilization at a certain level[11,14-18]. These dynamics are likely driven by an initial expansion of plasmablasts which produce large amounts of antibody but die off quickly followed by a slower decay of antibody titers (the half-life of IgG is approximately three weeks) which then transitions into a steady state level of antibody produced by long-lived plasma cells[19]. However, it is currently unknown, if the magnitude of the initial expansion of plasmablast and the associated antibody titers are correlated with the steady state level of serum antibody produced by long-lived plasma cells. This is an important question since steady state antibody levels may provide superior protection from re-infection[20,21].

Specifically, there is currently a paucity of information on the kinetics of antibody decay among health care workers (HCW). It is suspected that SARS-CoV-2 infections among HCW are usually asymptomatic or mildly symptomatic and frequently associated with either underreporting of symptoms or heterogenous PCR and/or serologic diagnostics leading to most of them going undetected or unrecognized[22]. A large cohort study of HCWs in the greater New York City (NYC) area showed a seroprevalence of SARS-CoV-2 antibodies of 13.7%[23]. Our own data of anti N antibody screening among HCW at a New York City public hospital in the Bronx following the first "surge" of COVID-19 in May 2020, found that SARS-CoV-2 seroprevalence was at 27%[24]. Understanding the longitudinal kinetics of SARS-CoV-2 antibody response and the effectiveness of commercial antibody measurement assays is crucial to correctly determine infection rates, sero-prevalence and true sero-reversion rates in both

infected and vaccinated individuals – and to better understand protection associated with sero-positivity.

In this study, we aimed to investigate the longitudinal humoral responses to viral N and the spike protein and to evaluate their correlation to clinical symptoms and baseline characteristics of the HCW cohort. We also evaluated if initial high antibody levels correlated with high antibody titers at steady state.

Methods

Study setting and population

This is a prospective longitudinal study done in two phases after receiving Institutional Review Board approval (IRB # 20-009). The Phase 1 study was conducted in May/June, 2020 and the Phase 2 was completed August/September 2020. The cohort included HCWs who worked at the New York City Public Hospital in the South Bronx. Information about the study was disseminated among health care workers via hospital's intranet bulletins, by research staff approaching on duty staff and handing out study flyers and introducing the study in multiple department meetings. The HCWs who had participated in Phase 1 were called individually to schedule an appointment with research staff for blood-work and survey completion for Phase 2 study.

In the Phase 1 of the study, after informed consent, participants underwent qualitative serology testing (Abbott Architect SARS-CoV-2 IgG Assay, Abbott Park, IL 60064 USA)[25] and a nasopharyngeal swab for SARS-CoV-2 (Bio-Reference Laboratories, Inc., Elmwood Park, NJ, USA). They also completed an initial online survey on demographics, symptoms of COVID-19 including duration, and healthcare/community exposure. An extra sample was collected and

stored at -80°C for subsequent analysis. These samples were processed using a quantitative enzyme-linked immunosorbent assay (ELISA), developed by Mount Sinai Health System (MSH ELISA)[26,27], that correlates well with virus neutralization, to detect RBD and full-length spike (S) reactive antibodies. In Phase 2 of the study, consenting HCWs underwent qualitative and quantitative serology assessment by Abbott and MSH ELISA tests, respectively. They also completed a follow-up online survey including information about demographics, interval SARS-COV2 PCR positivity and healthcare/community exposure.

Antibody assays

The Abbott Architect assay uses a qualitative chemiluminescent microparticle immunoassay technology targeting the N antigen of the virus with a reported sensitivity of 100% (CI 95.8–100%) and specificity of 99.6% (CI 99–99.9%)[25]. The MSH ELISA consists of an initial ELISA using serum or plasma to detect specific IgG against the RBD of SARS-CoV-2 at a single dilution, followed by quantitative titrations of presumptive positives in a confirmatory ELISA against full length SARS-CoV-2 spike protein (S)[28]. The positive result from the spike ELISA is reported as antibody at a titer of 1:80 or higher. Test performance assessment revealed that PCR+ samples were 94 % positive and all negative samples returned a negative result for 100% negative agreement[29].

Survey

The open-access online SurveyMonkey tool (SurveyMonkey, San Mateo, CA, U.S.A.; http://www.surveymonkey.com) was used to create and administer our survey to participating HCWs. The survey in both phases was developed with feedback from the Research Team. Open text questions were minimized. Preliminary versions of the survey were piloted among a focus group of 10 healthcare providers and their feedback about length, flow, ease of response,

and acceptability to respondents was incorporated to finalize the version administered to the participants. The online survey was accessed by a unique identification number assigned to each participant, blinded to the research team to ensure confidentiality. The Phase 1 survey was designed to capture demographics and current medical history, number and duration of symptoms of COVID-19 infection (exposure during the pandemic prior to Phase 1), domestic/international travel, and healthcare and community exposure[24]. The risk of exposure in the healthcare setting and community exposure was determined based on CDC guidelines[30]. The Phase 2 survey requested information on new comorbidities, persistent COVID-19 symptoms (cough, shortness of breath, anosmia, ageusia, myalgia, nausea, and/or diarrhea), interim testing via antibody and/or reverse transcription polymerase chain reaction (RT -PCR) (if present) and their result (positive/negative), presence of positive SARS-CoV-2 PCR results in the preceding months (exposure after Phase 1 sample collection), interim domestic/international travel, and continued use of personal protective equipment (PPE). Both surveys have been attached as online supplemental materials (Supplementary materials section).

Statistical analysis

Convenience sampling design was adapted to recruit participants with a goal of 500 participants. Descriptive statistics were used to summarize the baseline characteristics of the cohort and key study outcome variables. Categorical variables were compared by the Chi–squared test, while continuous variables were compared by a Student's t-test. The spike antibody titers were described as geometric means. Correlations were calculated using standard Pearson and Spearmen correlation. Multiple linear regression was applied to determine the predictors of log10 rate of decay from Phase 1 to Phase 2 of anti-spike antibodies. A p-value

of <0.05 was considered significant. All statistical analyses were performed using SPSS version 27 (IBM, USA).

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Results

For Phase 1 of the study, 500 healthcare workers underwent both PCR and serology testing. Of these, 137 were positive by for anti-N antibody (Abbott) and 142 were positive by the MSH ELISA. In the Phase 2, 178 participants from the initial cohort. The interval between Phase 1 and Phase 2 was 133±21 days. The details of patient enrolment are described in **Figure 1**. While 46 of the 178 tested subjects remained positive for the anti-N antibody (Abbott), 70 were positive by the MSH ELISA in the second phase. Anti -spike titers of the 5 subjects in the first phase were close to the cut off for positivity. Twenty-two subjects who were negative for anti-N antibody in Phase 2 had positive titers of anti-RBD and anti-spike antibodies, though lower than their Phase 1 levels. Among the subjects who participated in the Phase 1 and Phase 2 study, 68 were positive in both phases by the MSH ELISA, 110 were negative in both phases and 2 were positive only in Phase 2 with previously negative results in Phase 1.

The baseline characteristics of study participants who were positive by MSH ELISA in both phases (n=68) and those who were negative in both phases (n=108) are shown in **Table 1**. The mean age of the participants was 44.7±12.4 years, and 63.1% were female. Overall, 30.7% of the HCWs were Latinx, 29.5% were Asian, 16.5% were Black and 17.6% were White. COVID-19 related symptoms were present in 83.8% (57) of the subjects who were positive in both phases, while only 42.6% (46) of the subgroup who had negative antibodies in both phases

admitted to symptoms prior to Phase 1. The duration of symptoms prior to Phase 1 was longer among the symptomatic positive group (48.3% for >14 days) in comparison to symptomatic negative group (17.8% for >14 days). The mean duration of symptoms to Phase 1 testing in the symptomatic positive sub cohort was 47.9 ±16.0 days. Persisting symptoms of COVID-19 were reported in 19 (27.9%) subjects from the cohort with positive antibodies in both phases.

Clinical characteristics and seropositivity to spike protein in both phases

Table 2 describes the characteristics of the symptomatic and asymptomatic subjects who were positive for anti-spike antibody in both phases. Baseline characteristics were comparable between the groups and no difference either in the healthcare or community exposure or in the location of work (ED/Inpatient/intensive care unit, OR etc.) between the two groups was observed. Titers of anti-spike antibodies (geometric mean area under the curve (AUC)) were higher in symptomatic subjects than in asymptomatic positive subjects (6754 AUC vs. 5803 AUC) in Phase 1. However, in the Phase 2 analysis we observed marginally higher titers in the asymptomatic subgroup compared to the symptomatic subgroup (2383 AUC vs. 2198 AUC). Figure 2 illustrates the symptomatic and asymptomatic antibody levels of anti-spike antibodies. The rate of decay was higher in the symptomatic subgroup (geometric mean 32.96 per day) compared to the asymptomatic (geometric mean 23.42 per day) suggesting delayed antibody/kinetics in the asymptomatic cohort.

Phase 1 anti-spike antibody titer and clinical correlations

A Pearson's product and Spearman's rank-order correlation was run to assess the relationship between cohort characteristics including age, gender, comorbidities, number of symptoms of COVID-19, healthcare exposure and Phase 1 anti-spike titers in our cohort (**Figure 3**). One hundred-forty-three subjects with a positive test in Phase 1 were included in the analysis.

Scatter plot analysis showed a monotonic relationship between the variables. A statistically significant weak positive correlation was observed between age and Phase 1 anti-spike antibody titers (R=0.269, p<0.01). Moderate positive correlation was present between presence of fever (R=0.319, p<0.01), number of symptoms (R=0.310, p<0.01) and days of symptoms (R=0.434, p<0.01) and anti-spike antibody titer; and weak positive correlation was observed with upper respiratory symptoms (R=0.278, p<0.01) and gastrointestinal (GI) symptoms (R=0.204, p<0.05) with anti-spike antibody titers.

Correlation of rate of decay of anti-spike antibody titers from Phase 1 to Phase 2 and clinical characteristics

Results of Pearson correlation to assess the relationship between cohort characteristics including Phase 1 anti-spike antibody titers, age, gender, comorbidities, symptoms of COVID-19, number of symptoms of COVID-19, healthcare exposure and decay of anti-spike titers between the two phases in our cohort is shown in **Figure 4.** A strong positive statistically significant correlation was observed between Phase 1 titers and decay of anti-spike antibody titers between the two phases (R=0.898, p<0.001). Medium positive correlation was observed between presence of fever (R=0.428, p<0.001), GI symptoms (R=0.340, p<0.05), number of symptoms (R=0.357, p<0.01), duration of symptoms (R=0.469, p<0.001) with decay of anti-spike antibody titers between the two phases respectively.

A pairwise comparison was performed between rate of decay of anti-spike antibody titers and patient characteristics (**Figure 5**). Rate of decay by gender was comparable (male; 30.73 AUC/day vs. female;34.68 AUC/day, p=0.413). Asian (86.0 AUC/day) demonstrated a higher rate of decay compared with Whites (7.2 AUC/day) and Blacks (19.61 AUC/day) individuals;

while Latinx (47.28 AUC/day) race had higher rate of decay compared with White (7.2 AUC/day) individuals. Subjects with fever had a higher rate of decay than those who did not report fever (53.08 AUC/day vs.16.14 AUC/day, $p<0.01$). Similarly subjects with GI symptoms had a higher rate of decay than those without (55.81 AUC/day vs.21.94 AUC/day, $p<0.05$). Subjects with symptoms restricted to less than seven days demonstrated a lower rate of decay when compared with symptomatic subjects over 7-14 days (13.60 AUC/day vs. 36.12 AUC/day, $p<0.05$) and when compared with symptomatic subjects with more than 14 days (13.60 AUC/day vs. 59.72 AUC/day, $p<0.001$). This finding was statistically significant. No difference was found when degree of exposure (High/Moderate: 28.18 AUC/day vs. Mild: 34.78 AUC/day, $p=0.395$) or job role (physician: 29.57 AUC/day vs. nurse: 53.59 AUC/day vs. Other: 26.83 AUC/day; $p=0.361$) were compared to rate of decay.

Predictors of rate of decay from Phase 1 to Phase 2 of anti-spike antibodies

Multiple linear regression analysis to predict the rate of decay with respect to age, Bacillus Calmette Guerin vaccination, number of symptoms, and Phase 1 (log10) anti-spike antibody titers is shown in **Table 3**. On the basis of a linear regression model that included the participants age, history of BCG vaccination, total number of COVID-19 symptoms and the Phase 1 concentration of log 10 spike antibody titers, the estimated change (decay) was 23.6 AUC/day when age was centred at median (42.6 years), there was positive history of BCG vaccinations, the total number of COVID-19 symptoms were centred at a median of 4, and the geometric mean of the $\log_{10}$ spike antibody titer was 3.78.

Discussion

With the COVID-19 pandemic showing no signs of abating, healthcare workers at the epicentre are at risk of infection due to occupational exposure as well as community

exposure. Sero-surveillance is the foundation for determining the scale and rate of exposures. With a multitude of serological assays getting emergency use approval from FDA, interpretation of the results of these assays and their clinical significance remains challenging. It is critical to understand the timing of the antibody response for acute interpretation. Confidence in analytical specificity of the assay is a critical requirement in measurement of the specific antibody responses. Recent studies have confirmed that anti spike titers especially anti-RBD titers can serve as surrogates for virus neutralization[31,32]. The Abbott SARS-CoV-2 IgG assay that targets antibodies to the nucleoprotein has a reported specificity and sensitivity of greater than 99% at 14 days or more following symptom onset and these measurements are not indicative or correlated to virus neutralization titers[33]. In comparison, the MSH ELISA targets the full-length S protein including RBD, a major target for neutralizing antibodies and has demonstrated excellent correlation to virus neutralization[11,26]. Longitudinal measurements of antibody levels have revealed that anti-N and anti S IgG antibodies continue to increase until the third week post symptoms and an approach that combines the detection of both of these antibodies would precisely detect almost 100% of all infectious exposures[34]. In our study, the mean number of days after symptoms to testing in Phase 1 was 47 days suggesting a higher likelihood of accuracy of the utilized assay.

Longitudinal blood sampling among HCWs working at a public hospital which was at the epicentre of the pandemic in NYC allowed for analysis of kinetics of anti-S and anti-N antibody responses. At two months after the first surge of infections, anti-N antibodies were detected in 27% and anti-S antibodies in 28% of participating HCWs. After an interval of four months, it is not surprising to note that among the participants who returned, 26% remained positive for anti N antibodies, while 31% of the previously anti-N antibody positive

subjects tested negative in phase 2. On the other hand, a similar analysis of the anti-S antibodies levels, confirmed that all the previously positive retested subjects continued to remain positive, albeit with lower titers. That being said, we acknowledge that the decline of N antibodies in our cohort could be due to the Abbott assay being less sensitive to describe the dynamics of N antibodies over time compared to other assays, like Roche, Siemens and Diasorin. Muecksch et al. demonstrated in their longitudinal analysis of clinical serology assay performance among COVID-19 convalescents, that there is a difference in diagnostic performance among various serologic assays[32].

COVID-19 related symptoms were significantly associated with positive anti-spike antibodies in both phases, with a similar association with longer duration (>14 days) of symptoms. Previous studies have demonstrated a lower level of IgG response among patients without symptoms or with mild symptoms compared to those with severe and critical disease[35,36]. A comparison of symptomatic versus asymptomatic subjects who tested positive for anti-spike antibodies in both phases, confirmed that the rate of decay of anti-spike antibody titers were faster in the symptomatic cohort than the asymptomatic subjects, which was seen also in the anti-N antibody kinetics. We observed a faster decay in this group with a lower titer of anti-spike antibodies in Phase 2 compared to the asymptomatic cohort (though the difference was not statistically significant). This could additionally be supported by the finding of fever and GI symptoms contributing to faster decay. Similar results of decreasing neutralizing antibody titer in symptomatic than asymptomatic patients were observed by Choe et al.[37]

Positive correlations for age, presence of fever, upper respiratory symptoms, GI symptoms, total number and duration of symptoms was observed with increased levels of anti-spike titers at Phase 1. Similar results of neutralizing antibody titers were also observed by

Boonyaratanakornkit et al. wherein they showed higher levels of neutralizing antibody titers were significantly associated with male gender, older adults, higher disease severity and shorter interval from recovery[38]. Based on a linear regression model with age centred at median (42.6 years), positive history of BCG vaccination, the total number of COVID-19 symptoms centred at a median of 4, and the geometric mean of the log10 anti-spike antibody titer at 3.78, we observed that the rate of decay of these antibody titers was 23.6 AUC/day. Evaluation of other characteristics with rate of decay between Phase 1 and Phase 2 showed a faster reduction in titers in Asian participants and in those with fever and GI symptoms. A slower decrease was noted among patients with shorter duration (<7 days) of symptoms, with no other significant correlation noted with any other baseline demographics or clinical characteristics.

As described above, higher antibody titers are associated with a larger number of symptoms, longer duration of symptoms and – as described by others as well – disease severity in general. We also found that higher initial antibody titers were associated with faster antibody decay during the two time points. Initial antibody responses are driven by short lived plasmablasts, which decay after a few days after producing massive amounts of antibody. IgG has a relatively long half-life of approximately three weeks, but decay is inevitable since the plasmablasts initially producing it disappear. Usually, titers then drop until they reach relatively stable levels of antibody which are maintained by long-lived plasma cells in the bone marrow[19]. The two time points described in this study represent the initial peak response and likely the stable level after the initial decay. We found that individuals with higher initial titers had a faster decay rate during the observation period meaning the difference between peak and stable, long-lived antibody levels were larger. This indicates that there is likely no direct correlation between the magnitude of the initial expansion of plasmablasts and the number of long-lived plasma cells that migrate to the bone marrow. It is critical to recognize that steady state antibody titers are

similar between the symptomatic and asymptomatic subgroups, suggesting that mid-term humoral protection might be similar after infection regardless of disease severity.

Our study has the following limitations: First being a single center study with a small convenience sampling method that included a smaller number of participants in Phase 2 of the study. Following the pandemic, the HCWs who had volunteered from around the country were transferred back and thus lost to follow-up. While this did decrease the overall sample size, it is notable that the rates of positive and negative results remained proportional. Secondly, there is a possibility of recall bias in the participant's responses on the online survey. Lastly, the study findings can underestimate rates of prior infections based on timing of the testing given that antibodies are only transiently detectable following infection.

In conclusion, findings from this study are similar to other studies that have reported that higher magnitude of anti-spike titers may correlate with protection against reinfection, in spite of the observed decay in the antibody levels[20,21]. Nevertheless, further studies to evaluate the longevity of immunity, especially in context of widespread administration of spike-based vaccine among HCWs would be important in predicting herd immunity to COVID-19 infections.

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Contributions: V.M., M.A.S. and U.V. designed the study. J.M.C., M.A.S., B.Y., V.P.G. analysed the data. E.V. and M.G. assisted with participant follow-up and coordination with the assistance of A.P., U.V., V.M., and M.A.S. The Mount Sinai Health System team, J.M.C. and F.K., performed the measurements for anti-Spike and Anti-RBD antibodies. V.M., U.V. and A.P. were responsible for the clinical care of the research participants and supervised the day-to-day operation and coordination of the study by M.K., V.D., M.A.S., B.Y., V.P.G., M.G., E.V., and M.G. V.M. and F.K wrote the manuscript and are the guarantors of this work and have full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, with the assistance of J.C.Q., M.A.S., B.Y., V.P.G., and M.G. All authors critically revised the draft and approved the final manuscript.

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Competing Interests: F.K. is listed as a co-inventor on a patent application filed by The Icahn School of Medicine at Mount Sinai relating to SARS-CoV-2 serological assays and NDV-based SARS-CoV-2 vaccines. Mount Sinai has spun out a company, Kantaro, to market serological tests for SARS-CoV-2. F.K. has consulted for Merck and Pfizer (before 2020), and is currently consulting for Seqirus and Avimex. F.K.'s Krammer laboratory is also collaborating with Pfizer on animal models of SARS-CoV-2.

All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Patient and public involvement: Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication: Not required

Ethics approval: The study protocol was approved by the Institutional Review Board approval (IRB # 20-009, Lincoln Medical Center, Office of the Institutional Review Board approved as per 45 CFR 46 & 21 CFR50,56 under a full board committee and gave its approval on 4/28/2020). All participants provided written informed consent for the use of their data.

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Data availability statement: Data are available on request from the corresponding author. All data relevant to the study has been included in the article.

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Table 1: Broad characteristics among health care workers assessed for antibody reactivity to spike SARS-CoV-2 protein in Phase 1 and Phase 2

	Overall †	Spike ELISA (AUC) positive in both phases	Negative Reactivity to spike (AUC) in both phases	p value
	N=176	n=68	n=108	
Age, years	44.7+12.4	42.9+11.9	45.8+12.7	0.099
Female, Gender	111 (63.1%)	40 (58.8%)	71 (65.7%)	0.467
Race				0.666
Latinx	54 (30.7%)	21 (30.9%)	33 (30.6%)	
Asian	52 (29.5%)	18 (26.5%)	34 (31.5%)	
Black	29 (16.5%)	15 (22.1%)	14 (13.0%)	
White	31 (17.6%)	10 (14.7%)	21 (19.4%)	
Other	10 (5.7%)	4 (5.9%)	6 (5.9%)	
Comorbidities	54 (30.7%)	25 (36.8%)	29 (26.9%)	0.214
BCG vaccine received in childhood	87 (49.4%)	35 (51.5%)	52 (48.1%)	0.902
COVID-19 related symptoms prior to Phase 1	103 (58.5%)	57 (83.8%)	46 (42.6%)	<.001
Duration of symptoms				<.001
<7 days	48 (46.6%)	18 (31.0%)	30 (66.7%)	
7-14 days	19 (18.4%)	12 (20.7%)	7 (15.6%)	
>14 days	36 (35.0%)	28 (48.3%)	8 (17.8%)	
Time from symptom to positive result, days	45.7+19.9	47.9+16.0	42.9+24.1	0.062
RT-PCR positive result for SARS-CoV-2 prior to Phase 1	51 (29.0%)	49 (72.1%)	2 (1.9%)	<.001
RT-PCR positive result for SARS-CoV-2 during Phase 1	14 (8.0%)	13 (19.1%)	1 (0.9%)	<.001
Persisting symptoms from COVID-19	25 (14.2%)	19 (27.9%)	6 (5.6%)	<.001
Nature of work				0.306
Physicians	81 (46.0%)	29 (42.6%)	52 (51.5%)	
Nurses	29 (16.5%)	15 (22.1%)	14 (13.0%)	
Others	64 (36.4%)	24 (35.3%)	40 (39.6%)	
Hospital areas worked in:				
Emergency department/Inpatient units	118 (67.0%)	50 (73.5%)	68 (63.0%)	0.141
Ambulatory care/Clinics	72 (40.9%)	27 (39.7%)	45 (41.7%)	0.631
Administration/Non-clinical care areas	24 (13.6%)	9 (13.2%)	15 (13.9%)	0.867
Community exposure	47 (26.7%)	19 (27.9%)	28 (25.9%)	0.591

Household exposure	39 (22.2%)	17 (25.0%)	22 (20.4%)	0.343
PPE use at work	173 (98.3%)	67 (98.5%)	106 (98.1%)	0.226
Use of facemask outside of the hospital	158 (89.8%)	58 (85.3%)	100 (92.6%)	0.062

Continuous variables are expressed as mean (SD), categorical variables as n (%).
BCG, Bacillus Calmette–Guérin vaccine; PPE, personal protective equipment; RT-PCR, reverse transcription polymerase chain reaction.
† Demographic data is missing for 2 participants from the overall cohort.

Table 2: Broad characteristics among health care workers with positive antibody reactivity to SARS-CoV-2 spike in both phases

	Overall	Asymptomatic for SARS-CoV-2 infection	Symptomatic for SARS-CoV-2 infection	*p value*
	n=68	n=11	n=57	
Age, Mean (±SD)	42.9 (±1.45)	44.5 (±3.8)	42.6 (±1.6)	0.557
Female, n (%)	40 (58.8%)	6 (54.5%)	34 (40.4%)	0.502
Race				0.753
Latinx	21 (30.9%)	3 (27.3%)	18 (31.6%)	
Asian	18 (26.5%)	3 (27.3%)	18 (26.3%)	
Black	15 (22.1%)	3 (27.3%)	12 (21.1%)	
White	10 (14.7%)	2 (18.2%)	8 (14.0%)	
Other	4 (5.8%)	0 (0%)	4 (7.0%)	
Comorbidities				
Hypertension	13 (19.1%)	2 (18.2%)	11 (19.3%)	0.650
Diabetes	6 (8.8%)	0 (0%)	6 (10.5%)	0.332
COPD and asthma	13 (19.1%)	1 (9.1%)	12 (21.1%)	0.326
Number of symptoms, median (IQR)	-	-	4.0 (2.0-5.0)	
Length of symptoms				
<7 days	-	-	19 (33.3%)	
7-14 days	-	-	12 (21.1%)	
>14 days	-	-	26 (45.6%)	
Degree of HCW exposure				0.492
High and Moderate	16 (23.5%)	2 (18.2%)	14 (24.6%)	
Minor	52 (76.5%)	9 (81.8%)	43 (75.4%)	
Community exposure	19 (27.9%)	3 (27.3%)	16 (28.1%)	0.635
Household exposure	17 (25.4%)	3 (27.3%)	14 (24.6%)	0.557
Use of facemask outside of hospital	58 (85.3%)	9 (81.8%)	49 (86.0%)	0.722

Principal means of transportation				0.663
Public	33 (48.5%)	6 (54.5%)	27 (47.7%)	
Private	35 (51.5%)	5 (45.5%)	30 (52.6%)	
Nature of work				0.502
Physician	29 (42.6%)	4 (36.4%)	25 (43.9%)	
Nurse	15 (22.1%)	2 (18.2%)	13 (22.8%)	
Other	24 (35.3%)	5 (45.5%)	19 (33.3%)	
Hospital areas work in:				0.288
Emergency department/inpatient units	32 (47.1%)	6 (54.5%)	26 (45.6%)	
Ambulatory care/clinics	9 (13.2%)	2 (18.2%)	7 (12.3%)	
Impatient and outpatient setting	18 (26.5%)	3 (27.3%)	15 (26.3%)	
Administration/nonclinical care areas	9 (13.2%)	0 (0%)	9 (15.8%)	
Anti-spike reactivity (AUC)				
Reactivity in phase 1, G-Mean (IQR)	6590 (5165-8410)	5803 (2825-11920)	6754 (5177-8812)	0.647
Days from symptoms to first test, Mean (±SD)	-	-	47.7 (±1.9)	
Reactivity in phase 2, G-Mean (IQR)	2226 (1824-2718)	2382 (1494-3797)	2198 (1753-2755)	0.980
Days from symptoms to second test			174.5 (±4.1)	
Rate of decay, G-Mean (IQR)	31.14 (22.11-43.87)	23.42 (8.45-64.93)	32.96 (22.73-47.82)	0.382

Continuous variables are expressed as mean (SD) or interquartile range (IQR), categorical variables as n (%).

AUC, area under the curve; COPD, Chronic obstructive pulmonary disease; HCW, health care worker

Table 3: Multiple linear regression analysis of rate of decay for anti-spike antibodies between Phase 1 and Phase 2

Rate of decay (log10)	*B*	95.0% CI for B		*SE B*	ß	R^2	▲R^2
		LL	UL				
Model						0.83	0.82
Constant	-3.203**	-3.647	-2.759	.222			
Age (per 10-year change)	.014	-.005	.007	.002	.030		
BCG vaccination	.131**	.030	.310	.046	.121		
Number of symptoms	.013	-.029	.060	.012	.050		
ELISA reactivity (Log10)	1.159**	1.050	1.419	.059	.916		

*B: Un*standardized regression coefficient; CI: confidence interval; LL: lower limit; UL: upper limit; SE B: standard error of the coefficient; ß: standardized coefficient; R^2: coefficient of determination; ▲R^2: adjusted R^2.

***P<0.*05

BCG, Bacillus Calmette–Guérin vaccine

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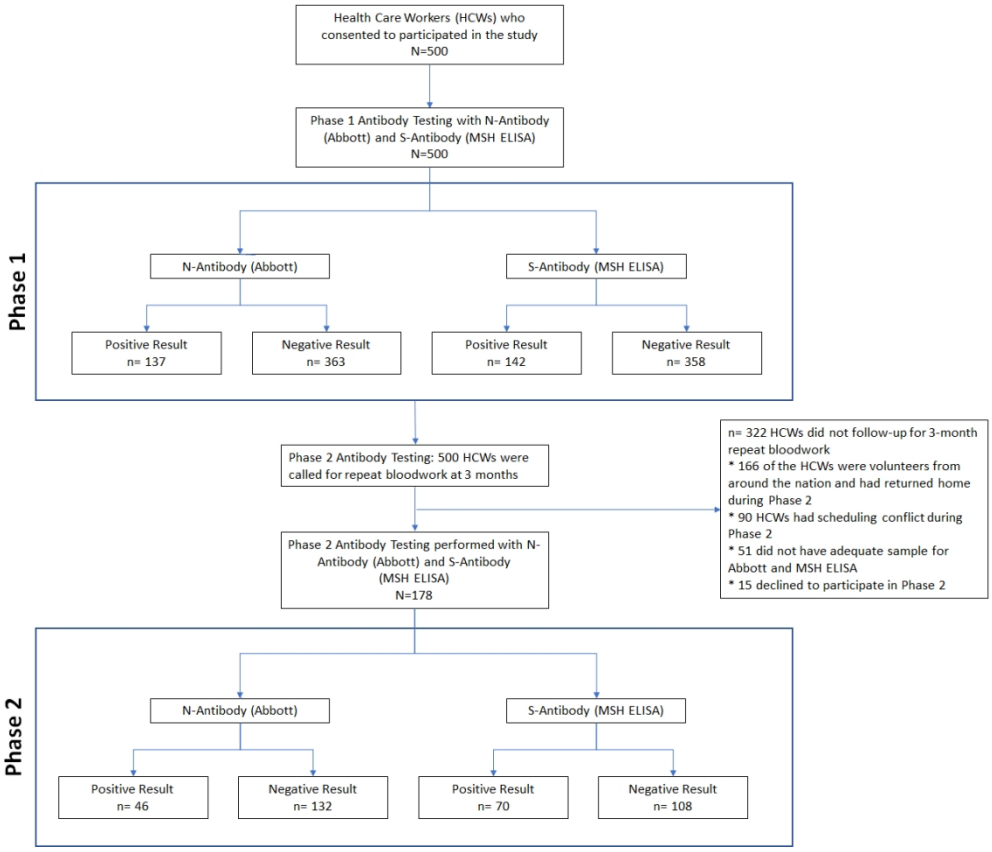
Figure 1: Flow Chart of patient enrollment, follow up and analysis

Figure 2: Antibody levels from Phase 1 in specimens obtained early during the pandemic (May 2020) and Phase 2 in follow up visit (August-October 2020) are shown for symptomatic and asymptomatic participants.

Figure 3: Simple correlation analysis of HCW with positive reactivity for anti- spike antibody in Phase 1 with baseline characteristics and symptoms

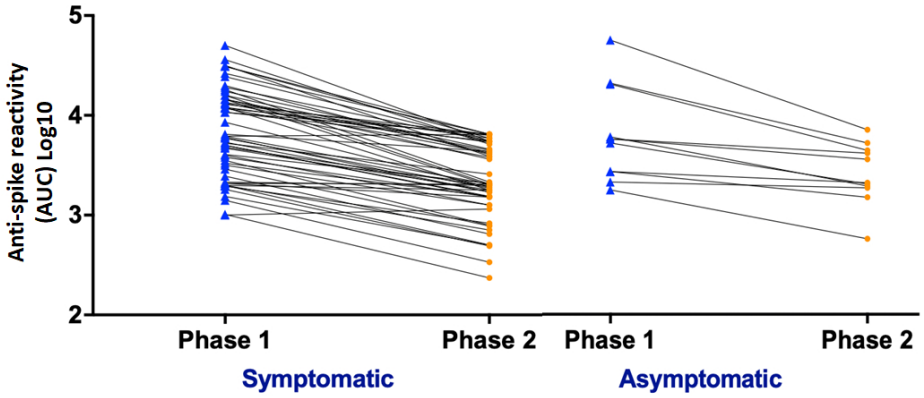
Figure 4: Simple correlation analysis of rate of decay of anti-spike antibodies between both phases with baseline characteristics and symptoms

Figure 5: Paired comparison between rate of decay of anti-spike antibody titers and patient characteristics



Flow Chart of patient enrollment, follow up and analysis

340x286mm (96 x 96 DPI)



Antibody levels from Phase 1 in specimens obtained early during the pandemic (May 2020) and Phase 2 in follow up visit (August-October 2020) are shown for symptomatic and asymptomatic participants.

295x129mm (96 x 96 DPI)

		ELISA reactivity in Phase 1 (Log10)	Age	Gender	Comorbidities	BCG vaccine	Fever	URS	GI symptoms	Number of symptoms	Days of symptoms	Degree of HCW exposure
ELISA reactivity in Phase 1 (Log10)	R	1										
	Sig.	-										
Age	R	0.269**	1									
	Sig.	0.001	-									
Gender	R	0.106	0.050	1								
	Sig.	0.209	0.552	-								
Comorbidities	R	0.130	0.468**	0.045	1							
	Sig.	0.123	0.000	0.596	-							
BCG vaccine	R	0.046	0.014	-0.129	0.084	1						
	Sig.	0.585	0.869	0.125	0.317	-						
Fever	R	0.319**	0.024	-0.089	0.018	0.060	1					
	Sig.	0.000	0.777	0.292	0.828	0.479	-					
URS	R	0.278**	0.039	0.059	0.069	-0.214*	0.464**	1				
	Sig.	0.001	0.645	0.486	0.413	0.010	0.000	-				
GI symptoms	R	0.204*	0.030	0.068	0.029	0.024	0.344**	0.291**	1			
	Sig.	0.015	0.723	0.423	0.733	0.775	0.000	0.000	-			
Number of symptoms	R	0.310**	-0.015	0.070	0.026	0.019	0.708**	0.665**	0.630**	1		
	Sig.	0.000	0.856	0.405	0.762	0.825	0.000	0.000	0.000	-		
Days of symptoms	R	0.434**	0.036	0.019	0.121	-0.038	0.422**	0.605**	0.347**	0.681**	1	
	Sig.	0.000	0.671	0.817	0.151	0.656	0.000	0.000	0.000	0.000	-	
Degree of HCW exposure	R	-0.145	-0.215**	-0.217**	-0.177*	0.017	0.017	0.034	0.000	0.039	-0.085	1
	Sig.	0.083	0.010	0.009	0.034	0.838	0.844	0.690	0.998	0.640	0.314	-

BCG, Bacillus Calmette–Guérin; URS, upper respiratory symptoms; GI, Gastrointestinal symptoms (nausea, vomit and diarrhoea); HCW, health care worker.
**. Correlation is significant at the 0.01 level., *. Correlation is significant at the 0.05 level.

Simple correlation analysis of HCW with positive reactivity for anti- spike antibody in Phase 1 with baseline characteristics and symptoms.

767x285mm (96 x 96 DPI)

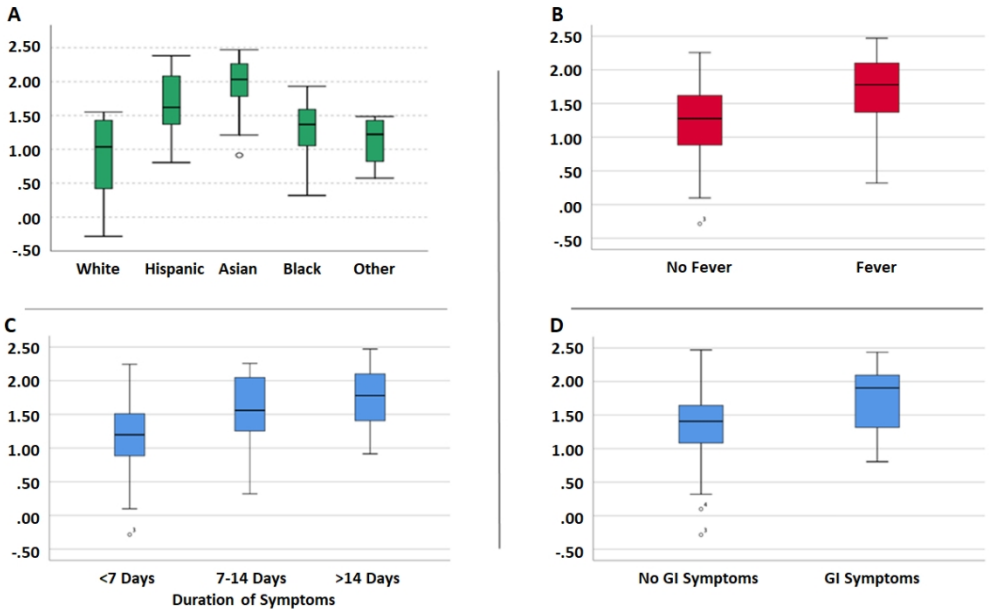
		Rate of decay (Log10)	ELISA reactivity (Log10)	Age	Gender	Comorbidity	Fever	GI symptoms	Number of symptoms	Duration of symptoms	Degree of HCW exposure
Rate of decay (Log10)	R	1									
	sig.	-									
ELISA reactivity (Log10)	R	0.898**	1								
	sig.	0	-								
Age	R	0.176	0.182	1							
	sig.	0.198	0.184	-							
Gender	R	0.044	0.094	0.001	1						
	sig.	0.751	0.493	0.994	-						
Comorbidity	R	0.193	0.156	0.514**	-0.092	1					
	sig.	0.158	0.257	0.000	0.502	-					
Fever	R	0.428**	0.463**	-0.090	-0.090	-0.122	1				
	sig.	0.001	0.000	0.515	0.512	0.374	-				
GI symptoms	R	0.340*	0.289*	-0.020	-0.072	-0.163	0.269*	1			
	sig.	0.011	0.033	0.885	0.603	0.234	0.047	-			
Number of symptoms	R	0.357**	0.360**	-0.256	0.008	-0.193	0.640**	0.555**	1		
	sig.	0.007	0.007	0.059	0.954	0.158	0.000	0.000	-		
Duration of symptoms	R	0.469**	0.469**	-0.049	0.123	0.142	0.204	0.207	0.430**	1	
	sig.	0.000	0.000	0.723	0.370	0.300	0.136	0.129	0.001	-	
Degree of HCW exposure	R	0.067	0.106	0.154	0.266*	0.202	-0.136	-0.075	-0.184	0.133	1
	sig.	0.625	0.439	0.261	0.050	0.140	0.321	0.586	0.180	0.334	-

BCG: Bacillus Calmette–Guérin; GI: Gastrointestinal symptoms (nausea, vomit and diarrhea); HCW: health care worker.

**. Correlation is significant at the 0.01 level, *. Correlation is significant at the 0.05 level

Simple correlation analysis of rate of decay of anti-spike antibodies between both phases with baseline characteristics and symptoms.

686x269mm (96 x 96 DPI)



Paired comparison between the rate of decay of anti-spike antibody titers and patient characteristics

213x133mm (144 x 144 DPI)

Supplementary Materials Section

Phase 1 Survey

Tital: Evaluation of Seroprevalence of Antibody to COVID-19 Virus among Healthcare Workers

IRB#20-009

Phase 1 - Survey

	Please enter the unique number that you were given:
	Age, years
	Gender
☐	Male
☐	Female
☐	Non-binary
☐	Prefer Not To Answer
	Do you have any of the following medical conditions?
☐	Hypertension
☐	Diabetes
☐	Heart failure
☐	COPD/Asthma
☐	Chronic kidney disease
☐	Cancer
☐	Rheumatic diseases (i.e. lupus, rheumatoid arthritis, etc)
☐	Not applicable
	Did you experience any of the following symptoms since May 1st 202 to present? (check all that apply):
☐	Fever
☐	Sore throat, cough, sinusitis
☐	Muscle aches, flu like symptoms
☐	Lack of taste
☐	Lack of smell
☐	Nausea/vomiting
☐	Diarrhea
☐	Non of the above
	When did you experience the above symptoms?
☐	May
☐	June
☐	July
☐	August
☐	NA - I dod not experience any of the above symptoms
	Approximate duration of symptoms (days)
☐	< 7 days
☐	7-14 days
☐	> 14 days

Phase 1 - Survey

☐	NA
	Did you get tested from May 1st to present with the following tests:
☐	SARS-CoV2 PCR (nasal swab)
☐	SARS-Cov2 Antibody (blood test)
☐	NA - I was not tested
	If you were tested, check the result that was positive:
☐	SARS CoV2 PCR (nasal swab) was positive
☐	SARS CoV2 Antibody (blood test) was positive
☐	NA
	Are you experiencing any new or persistent symptoms since March 2020? (check all that apply):
☐	Shortness of breath
☐	Chest pain
☐	Fever
☐	Palpitations
☐	Lack of tatse
☐	Lack of smell
☐	Headache
☐	Tingling or pricking sensation of hands/feet
☐	Chronically fatigued
☐	Decreased apetite
☐	No symptoms
	Did anyone living with you test positive for SARS-CoV2 PCR from May- August 2020?
☐	Yes
☐	No
☐	Not applicable.
	Did you travel in May- August 2020?
☐	Yes
☐	No
☐	Not applicable.
	Has there been a change in your living situation since May 2020?
☐	Moved from Apartment to Single Family Home
☐	Moved from Single Family Home to Apartment
☐	No change in living conditions
	Has there been a change in your commute to and from work since May 2020?
☐	Yes, I commute using public transportation now
☐	Yes, I commute using privatre transportaion now
☐	No change in the way I commute.

Tital: Evaluation of Seroprevalence of Antibody to COVID-19 Virus among Healthcare Workers

IRB#20-009

Phase 1 - Survey

	Have you been diagnosed with any new comorbidity(ies) in the last three months? (check all that apply):
☐	Diabetes
☐	Asthma
☐	COPD
☐	Coronary Artery Disease
☐	NA
	What PPE are you still using while seeing patients?
☐	N95 only
☐	N95 plus Surgical mask all the time
☐	N95 plus Surgical mask plus Eye protection
☐	N95 + Eye Protection + Face Shield While Seeing COVID-19 Positive Patients
☐	I don't use PPE

Supplementary Materials Section

Phase 2 Survey

Tital: Evaluation of Seroprevalence of Antibody to COVID-19 Virus among Healthcare Workers

IRB#20-009

Phase 2 - Survey

	Please enter the unique number that you were given:
	Do you have any of the following medical conditions?
☐	Hypertension
☐	Diabetes
☐	Heart failure
☐	COPD/Asthma
☐	Chronic kidney disease
☐	Cancer
☐	Rheumatic diseases (i.e. lupus, rheumatoid arthritis, etc)
☐	Not applicable
	Did you experience any of the following symptoms since May 1st 2020 to present? (check all that apply):
☐	Fever
☐	Sore throat, cough, sinusitis
☐	Muscle aches, flu like symptoms
☐	Lack of taste
☐	Lack of smell
☐	Nausea/vomiting
☐	Diarrhea
☐	Non of the above
	When did you experience the above symptoms?
☐	May
☐	June
☐	July
☐	August
☐	NA - I dod not experience any of the above symptoms
	Approximate duration of symptoms (days)
☐	< 7 days
☐	7-14 days
☐	> 14 days
☐	NA
	Did you get tested from May 1st to present with the following tests:
☐	SARS-CoV2 PCR (nasal swab)
☐	SARS-Cov2 Antibody (blood test)
☐	NA - I was not tested
	If you were you tested, check the result that was positive:
☐	SARS CoV2 PCR (nasal swab) was positive
☐	SARS CoV2 Antibody (blood test) was positive
☐	NA

Tital: Evaluation of Seroprevalence of Antibody to COVID-19 Virus among Healthcare Workers

IRB#20-009

Phase 2 - Survey

	Are you experiencing any new or persistent symptoms since March 2020? (check all that apply):
☐	Shortness of breath
☐	Chest pain
☐	Fever
☐	Palpitations
☐	Lack of tatse
☐	Lack of smell
☐	Headache
☐	Tingling or pricking sensation of hands/feet
☐	Chronically fatigued
☐	Decreased apetite
☐	No symptoms
	Did anyone living with you tested positive for SARS-CoV2 PCR from May - August 2020?
☐	Yes
☐	No
☐	Not applicable
	Did you travel in May - August 2020?
☐	Yes
☐	No
☐	Not applicable
	Has there been a change in your living situation since May 2020?
☐	Moved from Single Family Home to Apartment
☐	Moved from Apartment to Single Family Home
☐	No change in living conditions
	Has there been a change in your commute to and from work since May 2020?
☐	Yes, I commute using public transportation now
☐	Yes, I commute using privatre transportaion now
☐	No change in the way I commute.
	Have you been diagnosed with any new comorbidity(ies) in the last three months? (check all that apply):
☐	Diabetes
☐	Asthma
☐	COPD
☐	Coronary Artery Disease
☐	NA

Tital: Evaluation of Seroprevalence of Antibody to COVID-19 Virus among Healthcare Workers

IRB#20-009

Phase 2 - Survey

	What PPE are you still using while seeing patients?
☐	N95 only
☐	N95 plus Surgical mask all the time
☐	N95 plus Surgical mask plus Eye protection
☐	N95 + Eye Protection + Face Shield While Seeing COVID-19 Positive Patients
☐	I don't use PPE

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	#3	State specific objectives, including any prespecified hypotheses	7
Methods			

Study design	#4	Present key elements of study design early in the paper	7
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	7
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	7
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	8
Bias	#9	Describe any efforts to address potential sources of bias	16
Study size	#10	Explain how the study size was arrived at	9
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	7-9
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	9
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	9
Statistical methods	#12c	Explain how missing data were addressed	9
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	9
Statistical methods	#12e	Describe any sensitivity analyses	9

Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	9
Participants	#13b	Give reasons for non-participation at each stage	30
Participants	#13c	Consider use of a flow diagram	
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	9,23-24
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	**9,23-26**
9,23-26			
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	**4, 10**
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	**11**
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-11
Main results	#16b	Report category boundaries when continuous variables were categorized	10
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	**11**

Other analyses	#17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11-13
Discussion			
Key results	#18	Summarise key results with reference to study objectives	13-14
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	16
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	15-16
Generalisability	#21	Discuss the generalisability (external validity) of the study results	16-17
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18